

Notes on the jumping spider *Corythalia conferta* (Araneae: Salticidae), a possible myrmecophagous specialist in Argentina

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Abstract. We present data from a new study of *Corythalia conferta* Bayer, Höfer & Metzner, a recently described species that inhabits southern South America. A distribution update, morphological illustrations, and phylogeny of related *Corythalia* C. L. Koch species are presented. Also, we present data that suggest that this species may be the first *Corythalia* known to specialize in hunting ants (Formicidae). We describe its hunting behavior and habitat. During a 300 hour field study, we recorded 86 prey items, 98.8% of which were ants of 11 different species.

Keywords. Euophryini, hunting behavior, myrmecophagy, phylogeny, salticid, taxonomy

Introduction

Myrmecophagy is defined as *feeding on ants* and this has been recorded in many species within the family Salticidae (Cushing, 2012). Some of these species, including many orb-web spiders, are recognized as generalist ant eaters; they will feed on ants, but they are not specialized ant hunters. But other species like *Anasaitis canosa* (Walckenaer, 1837) can be defined as specialized ant eaters (Edwards et al., 1974, Jackson & Van Olphen, 1991). Ants are usually the most numerous potential prey for jumping spiders in many different environments, especially within tropical and subtropical regions. Ants have also developed formidable attack and defensive strategies like strong mandibles, stings or formic acid, and specialized ant eaters usually use adapted hunting techniques to avoid these risks. At the present time, there is no South American salticid species known to be a myrmecophagous specialist.

Here we present new data from a study of *Corythalia conferta* Bayer, Höfer & Metzner, 2020, a recently described and relatively common but poorly known species that inhabits southern South America. A distribution update, morphological illustrations and genetic descriptions are presented. Also, we present data that suggest that this species may be the first known *Corythalia* C. L. Koch spider specialized to hunt ants. We describe its hunting behavior, habitat selection and prey, based on 86 prey items.

Material and methods

Field surveys. Field observations of *Corythalia conferta* were made at the city of Puerto Iguazú, Misiones, Argentina. These spiders can be found on the wall in urban areas, where they build a shelter in small holes or concavities throughout the year, and adults are present mainly from October to April. We carried out periodic field surveys in four consecutive years (2014, 2015, 2016 and 2017) with a total of 300 hours of field work. We used two different survey strategies, active search on building walls for wondering individuals, and focal observations of individuals in their shelter. We made photographic records of spiders carrying their prey using a Nikon D3200 and a Nikon P900, and recorded with movies the strategy used to capture the prey when possible. Prey were collected and identified in the laboratory.

Taxonomy. Female genitalia were dissected as described by Levi (1965), and examined after digestion in a hot ~15% NaOH solution and subsequent clearing in clove oil to examine the internal structures. Clearing of the male palps was done by placing each palp in a ~15% NaOH solution and then transferring it to distilled water. Temporary preparations were observed and photographed using a Leica DM500 compound microscope and a Leica M60 stereomicroscope. Structures were sketched from incident light photograph models using a computer system for drawing and treatment of the image (Wacom digitizer tablet with GIMP, free software). Measurements were taken directly from a microscope ocular lens with an ocular micrometer and are expressed in millimeters. Photographs of live spiders were taken using Nikon D80 and D3400 digital cameras. Voucher specimens were deposited at the arachnological collection of the Instituto de Biología Subtropical, Misiones (IBSI-Ara, G. Rubio). Temporary preparations for analyses were coded as “GDR” or “CES”.

Mitochondrial DNA analysis. One leg of one specimen of each sex (indicated in the text as “tiss.s.”) was used for DNA extraction following a glass fiber-based extraction protocol (Ivanova et al., 2006), and was also deposited in the IBSI-Ara collection. A 658-bp fragment near the 5' end of the COI gene was amplified following standard protocols developed for DNA barcoding (Wilson, 2012) using primers C_LepFolF/C_LepFolR (Folmer et al., 1994) in the Barcoding Laboratory of the Museo Argentino de Ciencias Naturales (Buenos Aires), then sequenced in the Canadian Centre for DNA Barcoding (CCDB), Ontario, Canada. We compared the obtained sequences with all of the *Corythalia* sequences available in the BOLD and GenBank libraries. Sequences were edited using Chromas 2.6.5 (<https://technelysium.com.au/wp/chromas/>) and aligned using the MUSCLE algorithm (Edgar, 2004) in MEGA X (Kumar et al., 2018). We inferred the evolutionary history using the neighbor-joining method (Saitou & Nei, 1987); these analyses were conducted in MEGA X. The inferred bootstrap consensus tree was made with 10000 replicates (Felsenstein, 1985). Evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. We also inferred evolutionary history using the maximum likelihood method and the Tamura-Nei model (Tamura & Nei, 1993) with 1000 rapid bootstrap pseudoreplicates. The tree with the highest log likelihood (-2730.03) is shown in Figure 4. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with a superior log likelihood value. The tree in Figure 4 is drawn to scale, with branch lengths measured in the number of substitutions per site.

Results

Diagnosis and taxonomic description. The female (holotype from Instituto Butantan, São Paulo, IBSP 131451) and male (Paratype, IBSP 131451-I) *Corythalia conferta* were appropriately described in Bayer et al. (2020: 17, 18), not examined here. Supplemental illustrations of copulatory organs, chelicerae and habitus for this species are shown in Figures 1–3.

Genetic analysis. We obtained sequences from the CO1 gene for both a male and a female *C. conferta*. These sequences differ from any available *Corythalia* CO1 sequence, but they are almost identical within the series of *C. conferta* specimens from Argentina. A tree representing these differences is shown in Figure 4. Although this is a preliminary analysis, we can assume that both individuals belong to the same species, different from any other species of record in the BOLD and GenBank libraries. The male and female sequences for *C. conferta* are available in the Barcode of Life Data Systems (BOLD) with the code BOLD:ADR0351.

Distribution. Largely Argentinean Littoral and southeastern Brazil, presumably also in Uruguay.

Material examined. BRAZIL: Santa Catarina: Mariscal (-27.17131°, -48.50463°), 18 January 2015, G. D. Rubio coll., 1 female (IBSI-Ara 0272). ARGENTINA: Misiones: Iguazú: Puerto Iguazú (-25.753824°, -54.333741°), 11 September 2013, G. D. Rubio coll., 1 female (IBSI-Ara 0045; GDR 4035 for tiss.s.); same locality and collector, 2 October 2013, 1 male (IBSI-Ara 0059; GDR 4024), 1 November 2013, 1 female (IBSI-Ara 0084; GDR 4032); Parque Nacional Iguazú (-25.701336°, -54.444858°), May 2015, J. Baigorria coll., 1 male (IBSI-Ara 0413; GDR 0483); General Manuel Belgrano, Bernardo de Irigoyen (-26.253918°, -53.651332°), 17 December 2014, G. D. Rubio coll., 1 female (IBSI-Ara 0234); same locality and collector, March 2014, 1 subadult male (IBSI-Ara 0279; GDR 0446); Reserva Privada Karadya (-25.859584°, -53.960847°), 4 March 2015, J. Baigorria coll., 1 female (IBSI-Ara 0406); same locality and collector, August 2015, 1 male (IBSI-Ara 0541); San Pedro, Piñalito (-26.426761°, -53.840672°), 20 December 2014, J. Baigorria coll., 1 male (IBSI-Ara 0277; GDR 0445); 25 de Mayo, Colonia Aurora (-27.475942°, -54.526346°), 15 February 2015, G. D. Rubio coll., 1 female (IBSI-Ara 0342); Oberá (-27.48336°, -55.107168°), 5 February 2014, G. D. Rubio coll., 1 male (IBSI-Ara 0088; GDR 4030 for tiss.s.); same locality and collector, 30 January 2013, 1 male, (IBSI-Ara 0143; GDR 4069); same locality and collector, 21 February 2015, 1 male, (IBSI-Ara 0339); San Ignacio, Reserva Osununú, sector superior (-27.27983° -55.578087°), 9 December 2019, G. D. Rubio & C. E. Stolar coll., 1 male (IBSI-Ara 1404), 1 female (IBSI-Ara 1492); Leandro N. Alem, Cerro Azul, Estación Experimental INTA (-27.657515°, -55.437466°), 8 August 2017, G. D. Rubio coll., 1 male (IBSI-Ara 0965); same locality and collector, 22 January 2018, 1 female (IBSI-Ara 0980), 1 male (IBSI-Ara 0994), 21 November 2017, 1 female (IBSI-Ara 1004), 19 July 2018, 2 females (IBSI-Ara 1123); same locality, 29 June 2018, C. E. Stolar coll., 1 male (IBSI-Ara 1066); same locality and collector, 15 October 2018, 1 male (IBSI-Ara 1325; CES 0075), 20 September 2018, 1 male (IBSI-Ara 1327; CES 0035); same locality, 5 October 2018, G. D. Rubio & C. E. Stolar coll., 1 subadult female (IBSI-Ara 1273), 9 November 2018, 1 male (IBSI-Ara 1326; CES 0152); Chaco: Colonia Benítez, Estación Experimental INTA (-27.322934°, -58.956096°), 8 May 2018, G. D. Rubio coll., 1 male, 3 females (IBSI-Ara 1457); Corrientes: Paso de la Patria (-27.322299°, -58.57824°), 31 December 2014, G. D. Rubio coll., 2 males (IBSI-Ara 0240; GDR 0433).

Natural History. *Corythalia conferta* inhabits the Upper Paraná Atlantic Forest region and it can be found in modified forest, in crops like yerba mate and tea, and even on buildings in urban areas, probably ranging from southeastern Brazil to Central Argentina from the east. These spiders build a “tunnel-like” shelter with two circular to ovoid entrances from which they “ambush” their prey (Figure 5). They also search actively for prey in a wide area around their shelter. We have recorded adult males in shelters, but most of them were recorded wandering around and usually checking female shelters (for reproductive purposes probably). Of the 86 prey items recorded, 84 were ants from 11 different species, and only one was a mosquito (Diptera: Culicidae), and one was a rove beetle (Coleoptera: Staphylinidae). Prey ID and the number of captures recorded by prey species are shown in Table 1 and Figure 6. Smaller ants like *Pseudomyrmex* Lund were usually consumed by immature spiders while bigger ants like *Atta* Fabricius and some *Camponotus* (Mayr) were captured by adults and subadults only. We recorded 24 ant-hunting episodes. A photographic sequence taken from movie footage revealing the hunting technique is shown in Figure 5 and access to this complete video can be found at <http://hdl.handle.net/20.500.12123/7919>.

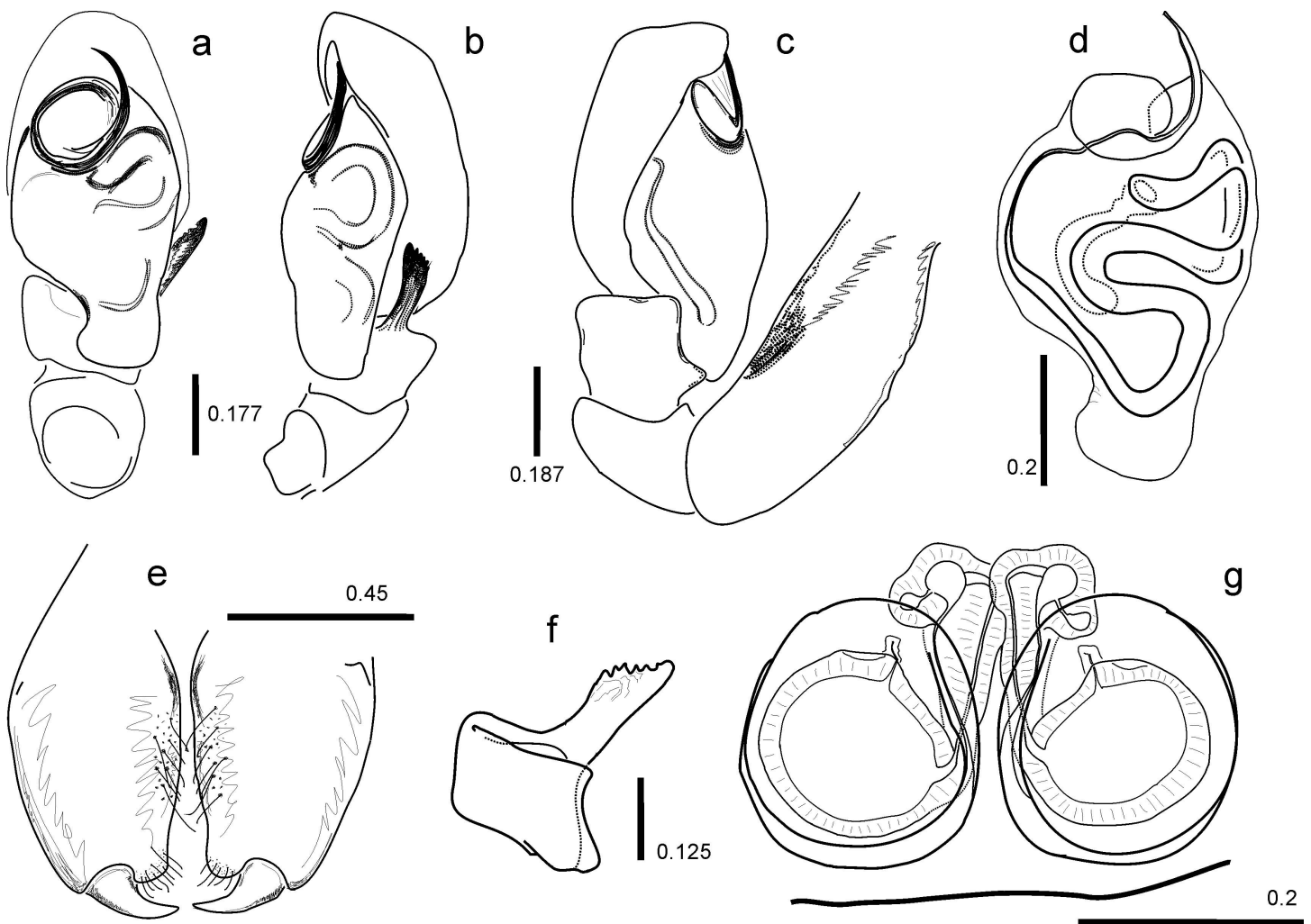


Figure 1. *Corythalia conferta* Bayer, Höfer & Metzner. **a**, Left palp (male IBSI-Ara 0088), ventral view. **b**, Same, retrolateral view. **c**, Same, prolateral view. **d**, Copulatory bulb (male IBSI-Ara 0277), cleared ventral view. **e**, Chelicerae (male IBSI-Ara 1326), anterior view. **f**, Tibia of palp (IBSI-Ara 0277), prolateral view. **g**, Female (IBSI-Ara 0045) cleared epigyne, ventral view. All measurements in mm.

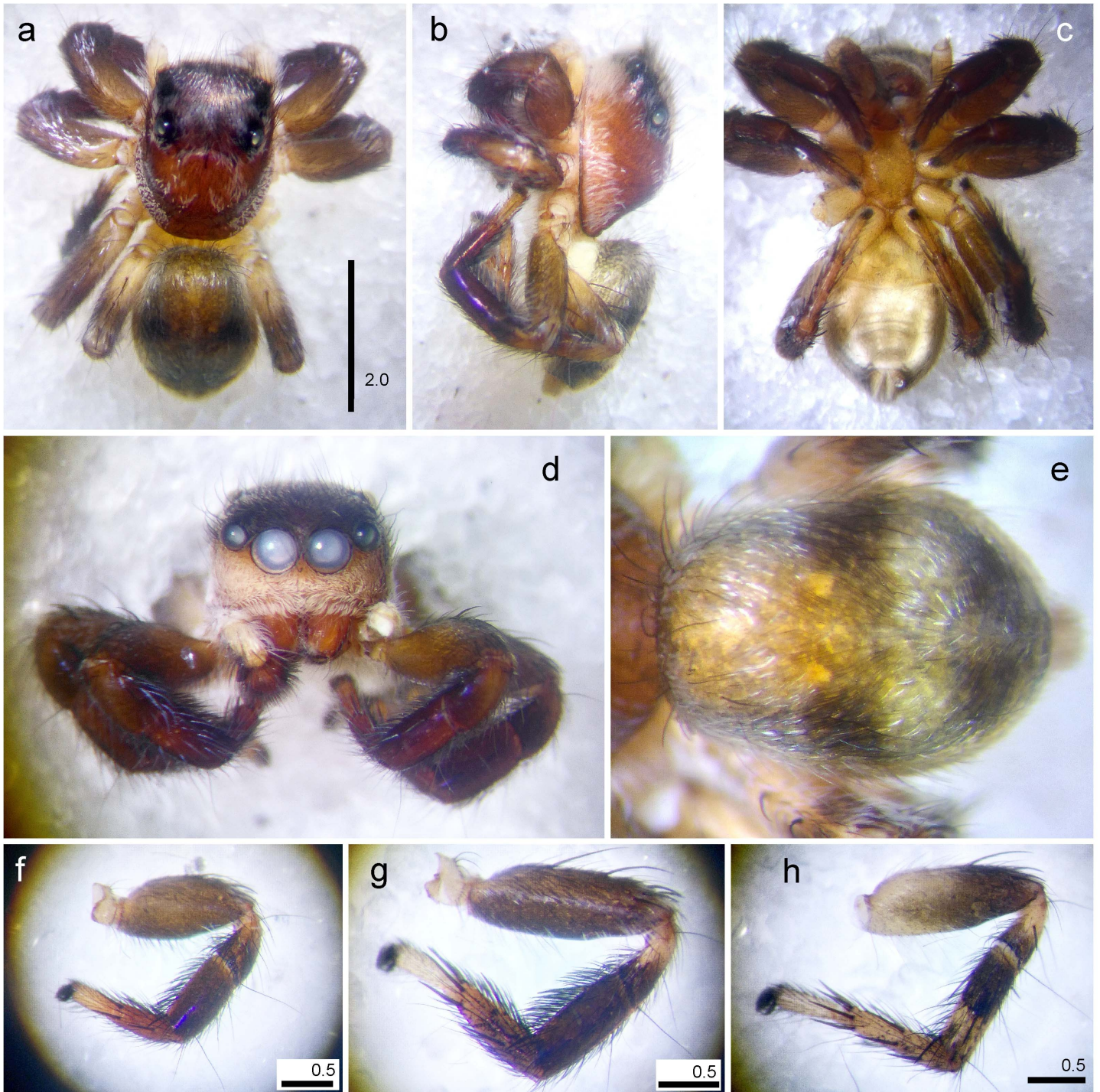


Figure 2. Male *Corythalia conferta* Bayer, Höfer & Metzner. **a**, Habitus (IBSI-Ara 0088), dorsal view. **b**, Same, lateral view. **c**, Same, ventral view. **d**, Same, anterior view. **e**, Abdomen, dorsal view. **f**, Leg II (IBSI-Ara 1326), anterior view. **g**, Leg III, anterior view. **h**, Leg IV, anterior view. All measurements in mm.

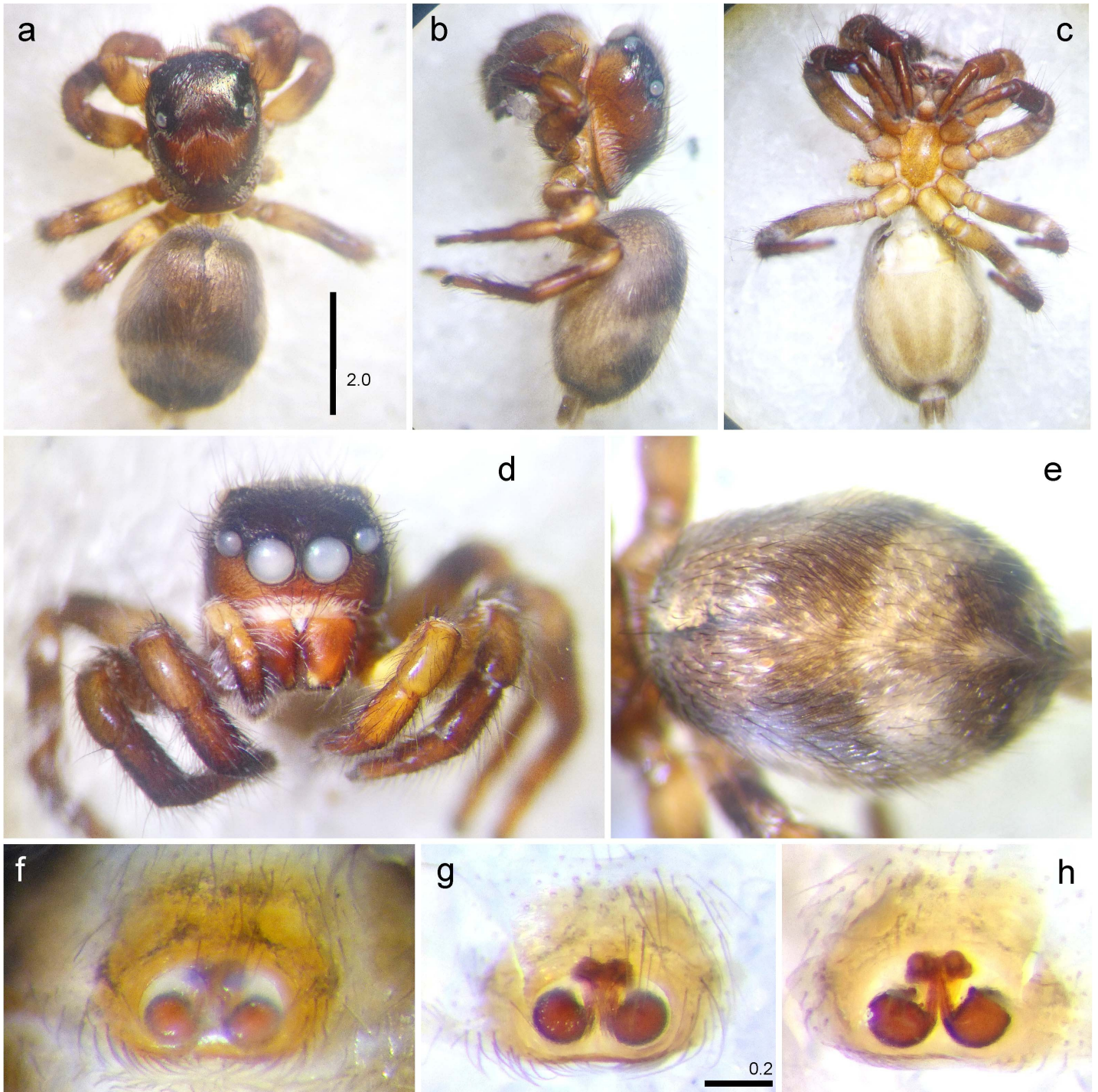


Figure 3. Female (IBSI-Ara 0045) *Corythalia conferta* Bayer, Höfer & Metzner. **a**, Habitus, dorsal view, **b**, Same, lateral view. **c**, Same, ventral view. **d**, Same, anterior view. **e**, Abdomen, dorsal view. **f**, Epigyne, ventral view. **g**, Same, cleared, ventral view. **h**, same, cleared, dorsal view. All measurements in mm.

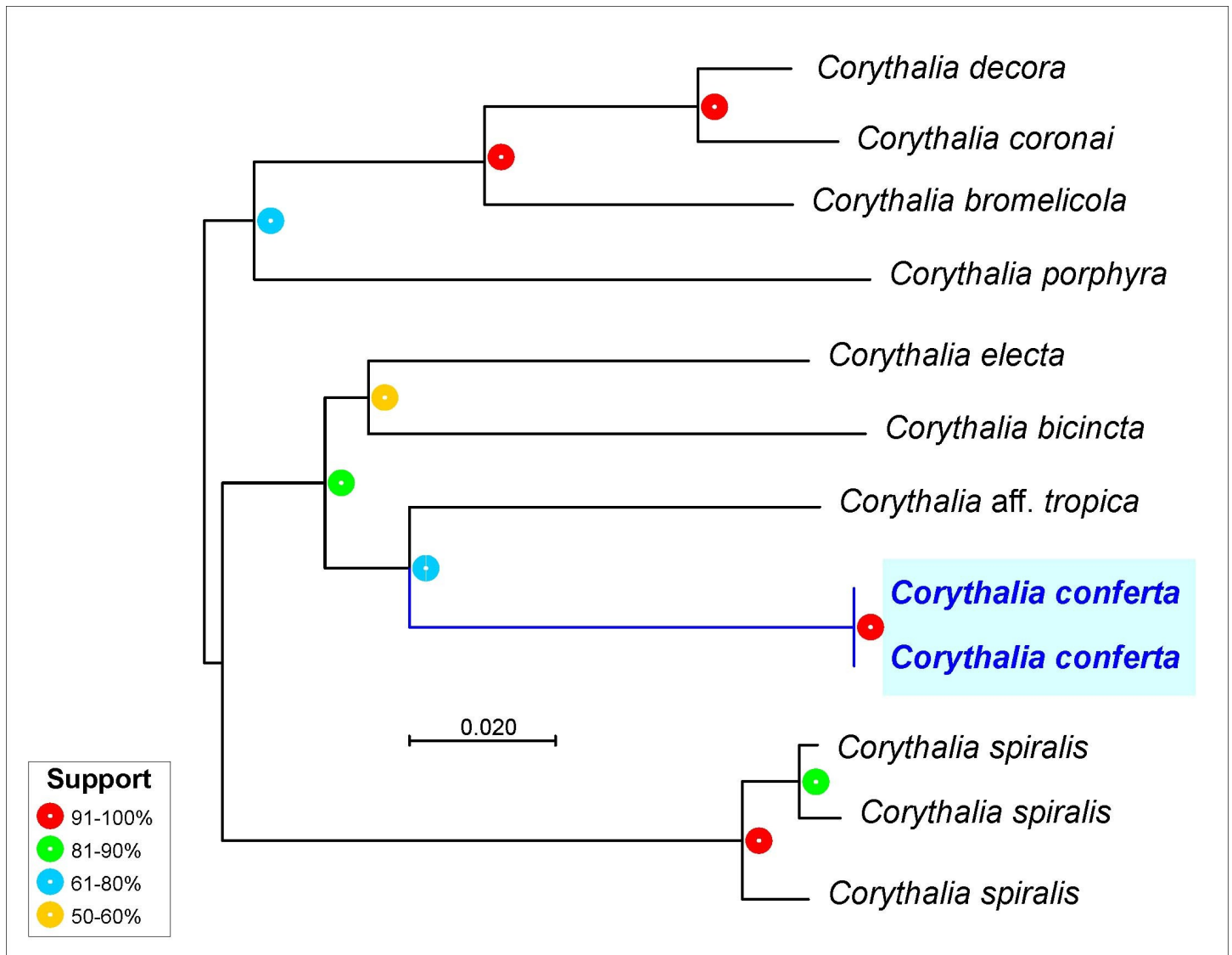


Figure 4. Hypothetical phylogeny of nine *Corythalia* species based on the neighbor-joining method for analysis of molecular data (barcode sequences).

In 22 of these episodes, the *Corythalia conferta* used the same technique to capture ants, attacking head-on, biting and holding the ant from the dorsal thorax near the pedicel in a single strike (movie minute 0:07). Immediately after this, each spider forced the ant into a “secure position,” pushing the head and thorax of the ant upside-down against the ground, facing the edge of the abdomen (Figures 7a, c, h, i). This position kept both ant mandibles and stings (or acid sprays) away from the spider. In six episodes, the ants were moving away from a spider that circled the ant and then attacked head-on. The spider venom acted quickly and the ants were paralyzed in less than 1 minute. Once an ant was captured, the spider carried the ant to its shelter and consumed it there. In one episode, a spider attacked the ant head-on, but from an upper position on a vertical wall. In doing this, the spider was not able to touch the wall (while the ant was standing on that wall), but remained suspended from its dragline while biting the ant in the usual place, retracting all of its legs against its body. Once the venom started to act (after a few seconds), the spider extended its legs and forced the ant into the “secure position.” On one occasion, an immature spider attacked a small ant (*Solenopsis* Westwood) from the side, repeating the usual pattern after that. The time required for *C. conferta* venom to completely paralyze ants varied among prey species, from around 30 seconds in *Atta* and *Solenopsis* to less than 3 minutes in *Camponotus*.



Figure 5. Selected sequential (1-28), but not consecutive, frames from a video record of hunting behavior by a female *Corythalia conferta* as it captured a *Camponotus* (Mayr) ant. The complete video can be seen at <http://hdl.handle.net/20.500.12123/7919>.

Table 1. Prey ants and other insects captured and consumed by *Corythalia conferta* during this study.

prey species	captures (N)	% of total
Family Formicidae		
<i>Atta</i> Fabricius + <i>Acromyrmex</i> Mayr ^a	12	13.9
<i>Camponotus</i> (Mayr) sp. 1	7	8.0
<i>Camponotus</i> sp. 2	12	13.9
<i>Camponotus</i> sp. 3	3	3.0
<i>Cephalotes</i> Latreille	14	16.3
<i>Pseudomyrmex</i> Lund sp. 1	10	11.7
<i>Pseudomyrmex</i> sp. 2	7	8.0
<i>Odontomachus</i> Latreille	9	10.6
indet. sp. 1	5	6.0
indet. sp. 2	5	6.0
Family Culicidae		
indet. sp. 1	1	1.3
Family Staphylinidae		
indet. sp. 1	1	1.3
TOTAL	86	100.0

^a identified in Figure 6 as *Leaf-cutting ants*

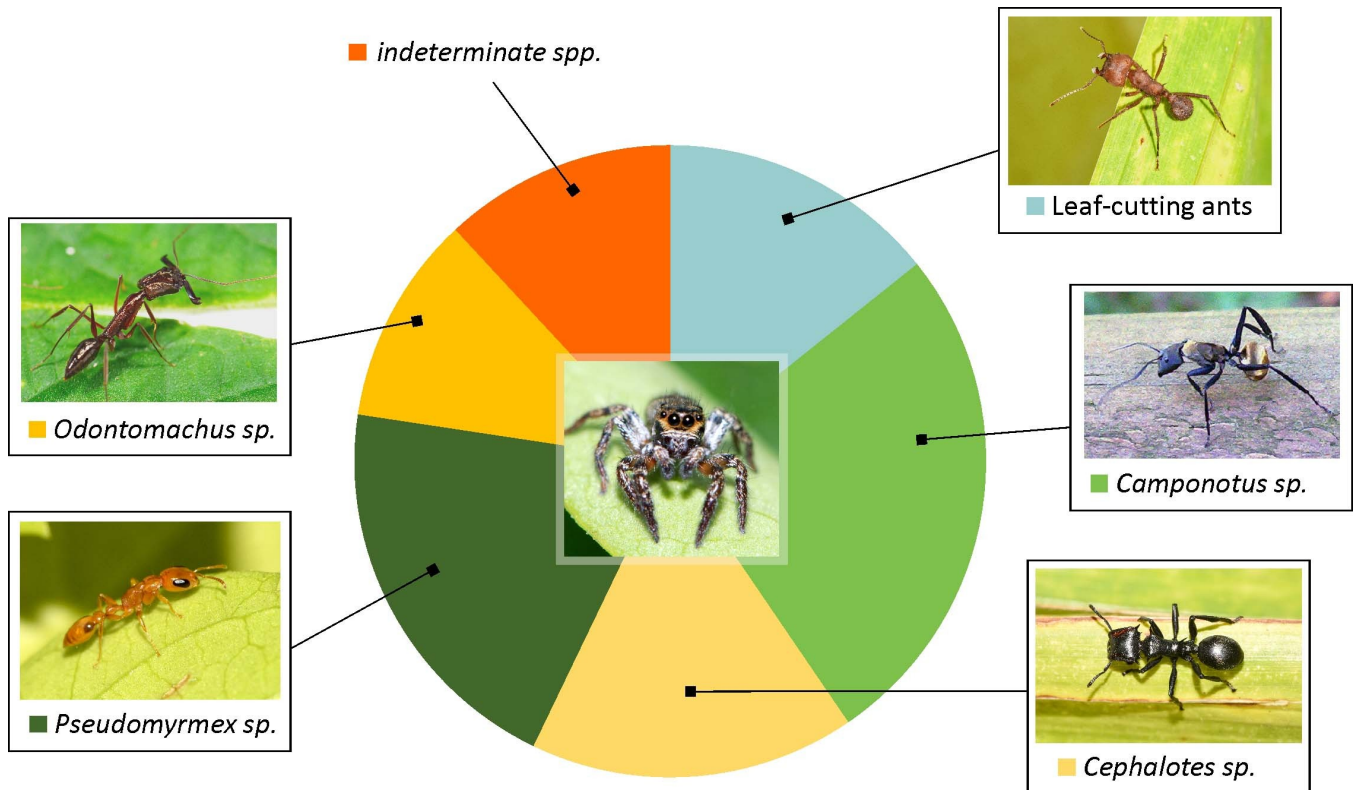


Figure 6. Pie chart depicting proportion of each type of prey ant captured and consumed by *Corythalia conferta* in this study. The “Leaf-cutting ants” are represented by ants of the genera *Atta* Fabricius and *Acromyrmex* Mayr.

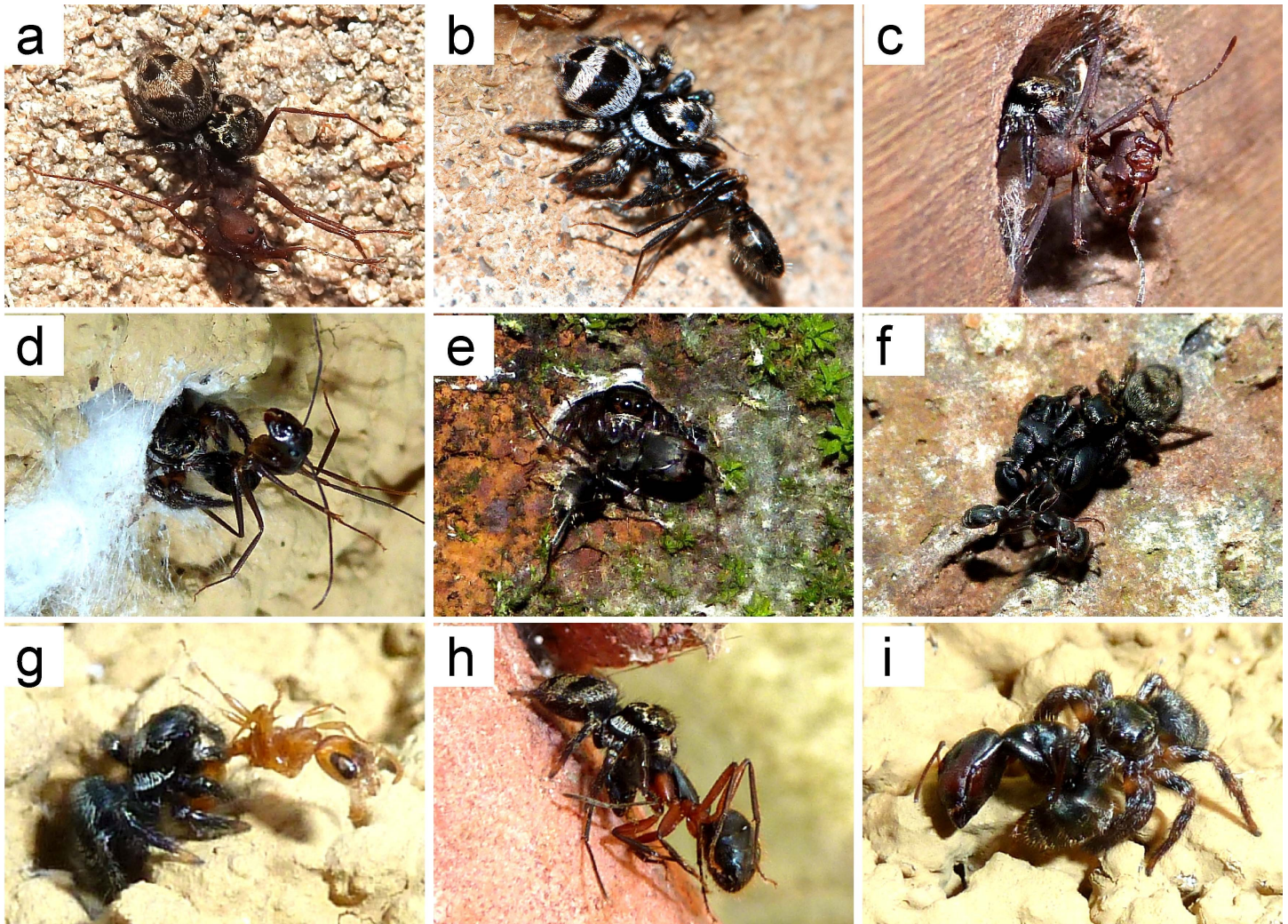


Figure 7. Jumping spiders *Corythalia conferta* in nature preying on different ants. **a,c**, *Atta* Fabricius. **b,d**, Indeterminate ants. **e,i**, *Camponotus* (Mayr) sp1. **f**, Mix of *Cephalotes* Latreille and *Pseudomyrmex* Lund sp2. **g**, *Pseudomyrmex* sp2. **h**, *Camponotus* sp3.

Two other salticid species, *Marma nigratarsis* (Simon) and *Balmaceda nigrosecta* Mello-Leitão, were frequently recorded on the walls inhabited by *Corythalia conferta*. *M. nigratarsis* was always wandering on walls actively looking for prey, while *B. nigrosecta* built shelters and stalked prey from these shelters, much like *C. conferta*. *M. nigratarsis* is the smallest of the three and both *B. nigrosecta* and *C. conferta* are about the same size. *C. conferta* never tried to capture either of the other salticids when they got close to their shelters, and we have never recorded any *M. nigratarsis* or *B. nigrosecta* feeding on either *C. conferta* immatures or adults. In one instance an adult female *M. nigratarsis* started to chase an immature *C. conferta*, which immediately reacted by turning to face the *M. nigratarsis*. After this, the *M. nigratarsis* stopped its hunting behavior and started to move away from the immature *C. conferta*. In another instance, an adult female *M. nigratarsis* moved directly to the shelter of an adult male *C. conferta*. When this female was less than 5 cm from the shelter, the male *C. conferta* elevated its first pair of legs and started moving from side to side. After this, the smaller *M. nigratarsis* immediately moved away from the male *C. conferta*. We recorded *B. nigrosecta* spiders feeding on *M. nigratarsis* on three different occasions. Although we have never recorded *B. nigrosecta* feeding on *C. conferta*, the much larger size of the adult *B. nigrosecta*, compared to the smaller immature *C. conferta*, as well as the proven ability of *B. nigrosecta* to capture wandering salticids, suggest that *B. nigrosecta* may be a predator of *C. conferta* in the study area.

In this study, and in other studies involving *Marma* and *Balmaceda* (e.g. Rubio et al., 2016), these species have never been recorded feeding on ants, and they always presented aversion to this family, either moving away from them (*Marma*) or hiding within their shelters (*Balmaceda*).

Adult and immature *Corythalia conferta* can be found throughout the year. Adults are more abundant from October to April.

Discussion

Although controlled experiments in laboratory conditions like the one proposed by Jackson & Van Opel (1991) or Li et al. (1996) could be performed to confirm *Corythalia conferta* preference for ants, fully 98.8% of the 86 recorded prey items in this field study of *C. conferta* were ants (Formicidae), suggesting a strong preference for ants by these spiders. It should be noted that, as observed with the related ant-eating euophryine *Anasaitis canosa* (Walckenaer, 1937), preferred prey in a laboratory setting can be quite different from those observed in nature (D. E. Hill, pers. comm.).

The idea that *C. conferta* are ant-eating specialists is supported by their use of specialized hunting techniques to capture ants, similar to the techniques described for *Anasaitis canosa* by Edwards et al. (1974), including a very efficient way of holding the ant to avoid both mandibles and stings or acid spray once it is captured. This unique behavior (the “secure position”) has never been recorded for other myrmecophagous salticids and it allows *C. conferta* to prey on at least 11 different ant species, even those with a formidable defensive structure like *Odontomachus* Latreille, or the large-bodied *Atta* species. In one opportunity, a *C. conferta* modified this behavior, apparently an adaptation to avoid risk. After landing in an unfavorable position it retracted all of its legs until its venom started to take effect, subsequently forcing an ant into the “secure position.” *C. conferta* in their shelters responded to ants as far as 50 cm away, starting to pursue them immediately. This suggests that either the shape or movement of ants triggers their hunting behavior (See also Jackson & Van Olphen, 1991; Jackson et al. 1998).

The time required for *C. conferta* venom to paralyze an ant appears to be, on average, less than that needed by other myrmecophagous salticids. For example, Edwards et al. (1974) recorded that *Anasaitis canosa* venom needed at least 3 minutes to paralyze different species and Jackson et al. (1998) describe an average time of 3 minutes for *Siler* Simon. There was no “stalking” approach or crouch seconds before jumping towards the prey as described by Forster (1977) for other Salticidae. As soon as a *C. conferta* reached the “head-on” position and the right distance it immediately jumped on the ant.

In several instances, we recorded *C. conferta* trying to capture insects other than ants, but they usually failed to do this. This may be because these spiders used the same technique that they used to capture ants, moving directly toward the prey instead of “stalking” it at a close distance to avoid detection, as other salticids would have done when pursuing flying insects. This might reflect specialization by *C. conferta* in the direction of myrmecophagy, perhaps between categories 4 and 5 according to Huseynov et al. (2008) in Cushing (2012), that is, between “facultative ant choosers that prefer ants to other prey” and “obligatory ant choosers that feed exclusively on ants.” Ants are fast-moving prey, and specialization of the hunting pattern (Forster, 1977) might increase the possibility of capture.

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References

- Bayer, S., H. Höfer, and H. Metzner. 2020.** Revision of the genus *Corythalia* C. L. Koch, 1850, part 1: diagnosis and new species from South America (Araneae: Salticidae: Salticinae: Euophryini). *Zootaxa*, 4806: 1-144.
- Cushing, P. E. 2012.** Spider-ant associations: an updated review of myrmecomorphy, myrmecophily, and myrmecophagy in spiders. *Psyche* 2012: 1-23.
- Edgar, R. C. 2004.** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792-1797. pmid:15034147
- Edwards, G. B., J. F. Carroll and W. H. Whitcomb. 1974.** *Stoidis aurata* (Araneae: Salticidae), a spider predator of ants. *Florida Entomologist*, 57: 337-346.
- Felsenstein, J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- Folmer, O., W. R. Hoeh, M. B. Black and R. C. Vrijenhoek. 1994.** Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. *Molecular Marine Biology and Biotechnology* 3: 294-299.
- Forster, L. M. 1977.** A qualitative analysis of hunting behaviour in jumping spiders (Araneae: Salticidae). *New Zealand Journal of Zoology* 4: 51-62.
- Huseynov, E. F., R. R. Jackson and F. R. Cross. 2008.** The meaning of predatory specialization as illustrated by *Aelurillus m-nigrum*, an ant-eating jumping spider (Araneae: Salticidae) from Azerbaijan. *Behavioural Processes* 77: 389-399.
- Ivanova, N. V., J. R. Dewaard and P. D. N. Hebert. 2006.** An inexpensive automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6: 998-1002.
- Jackson, R. R. and A. Van Olphen. 1991.** Prey-capture techniques and prey preferences of *Corythalia canosa* and *Pystira orbiculata*, ant-eating jumping spiders (Araneae, Salticidae). *Journal of Zoology* 223: 577-591.
- Jackson, R. R., D. Li, A. T. Barrion and G. B. Edwards. 1998.** Prey-capture techniques and prey preferences of nine species of ant-eating jumping spiders (Araneae: Salticidae) from the Philippines. *New Zealand Journal of Zoology* 25: 249-272.
- Kimura, M. 1980.** A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111-120.
- Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura. 2018.** MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547-1549.
- Levi, H. W. 1965.** Techniques for the study of spider genitalia. *Psyche*, 72: 152-158.
- Li, D., R. R. Jackson and B. Cutler. 1996.** Prey-capture techniques and prey preferences of *Habrocestum pulex*, an ant-eating jumping spider (Araneae, Salticidae) from North America. *Journal of Zoology* 240: 551-562.
- Rubio, G. D., J. E. Baigorria and G. B. Edwards. 2016.** First description of the female of the jumping spider *Balmaceda nigrosecta* Mello-Leitão (Salticidae, Dendryphantini, Marpissina). *ZooKeys* 563: 11-19.
- Saitou, N. and M. Nei. 1987.** The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.
- Tamura, K., and M. Nei. 1993.** Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512-526.
- Wilson, J. J. 2012.** DNA barcodes for insects. In: *DNA barcodes: Methods and Protocols* (ed. W. J. Kress and D. L. Erickson), Springer, pp. 17-46.