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Together but not intertwined: differences in sexual behavior between two sympatric and synchronic spider species, including one new synonymy (Araneae: Tetragnathidae: *Tetragnatha*)

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Abstract. Species recognition and reproductive isolation are critical for organisms to prevent expensive and unsuccessful matings. This may be particularly important in closely related species that coexist synchronously in the same habitat, and for which reproductive barriers are not entirely effective. *Tetragnatha argentinensis* Mello-Leitão, 1931 and *T. nitens* (Audouin, 1826) are two long-jawed orb weaver spiders whose feeding grounds and reproductive phenology overlap extensively. Since general patterns of sexual behavior observed in the field showed no apparent differences between these two species, we proposed to evaluate the occurrence of heterospecific mating, and explored the occurrence of potential reproductive isolation mechanisms between them by analyzing in fine scale the mating behaviors of each species and sex. We observed only one heterospecific mating, and few or no sexual interactions occurred in other crossed trials. We found that both species showed similar general mating patterns, however, there are some subtle differences between them. In *T. nitens*, males clasped the female's chelicerae with their own, but the opposite occurred in *T. argentinensis*. Moreover, males of *T. nitens* produced fewer hematodochal inflations, lower number of flubs, and shorter insertions than males of *T. argentinensis*. Females of *T. argentinensis* vibrated their abdomen at a higher rate. Our results indicate that these sympatric *Tetragnatha* species have successful reproductive isolation that probably takes place through recognition mechanisms occurring prior to mating. We provide an update on the taxonomic status of *T. argentinensis* and its distribution, and a new junior synonymy.

Keywords: Long-jawed spiders**,** sympatric species, reproductive isolation, pre-mating recognition

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Sympatric sibling species allow us to test hypotheses on the mechanisms involved in reproductive isolation. In an extensive number of species for which isolating reproductive barriers are not entirely effective, the reproductive interference (e.g., interacting sexually and interbreeding) is often common (Gröning et al. 2007; Bath et al. 2012). These interactions result in costs for heterospecifics, for example in time, energy, and gamete investment, as well as risk of damage or predation (Gröning & Hochkirch 2008). To counteract these high costs, selection has favored the evolution of a whole set of mechanisms, operating at different levels, to prevent interbreeding (Mayr 1963; Saetre et al. 1997; Pfennig & Pfennig 2009; Crampton et al. 2011; Bath et al. 2012). Sympatric species with similar phenotypes may reduce interbreeding by occupying different micro-habitats or temporal activity (Mayr 1963; Schoener 1974; Colwell & Fuentes 1975; Khelifa et al. 2013). Differences in genitalic morphology (genital mismatch) and courtship behavior could also prevent interbreeding if other factors do not limit heterospecific encounters (Eberhard 1985; Williams & Mendelson 2014; Zuk & Simmons 2018). Finally, postzygotic isolation could take place when the offspring is inviable, or when genetic incompatibility occurs (Williams & Mendelson 2014; Zuk & Simmons 2018).

Sexual behavior is essential for the occurrence or reinforcement of reproductive isolation (West-Eberhard 1983; Panhuis et al. 2001; Ritchie 2007; Fowler-Finn et al. 2019). Dramatic behavioral variation (e.g., absence of complete behavioral units), or subtle variation in sexual behaviors between populations (e.g., variation in the duration of behavioral patterns), can have significant evolutionary consequences in species divergence (Fowler-Finn et al. 2019). Although reproductive behavior is an important isolation mechanism in numerous species, it may not be an entirely effective barrier in recently diverged species (Pombi et al. 2017). In this scenario, it is expected that selection favors evolution of different isolation mechanisms to avoid heterospecific crosses (Coyne & Orr 1998; Ritchie 2007; Bath et al. 2012; Williams & Mendelson 2014).

In this context, spiders are a promising group to explore different aspects of isolation barriers (see Masta & Madisson 2002; González et al. 2015; Michalko & Pekar 2015). Courtship in spiders involves the use of multiple channels, including the exchange of chemical, visual, vibratory, and/or tactile signals, and the interaction between specific morphological structures (Huber 2005; Foelix 2011; Uhl & Elias 2011). Even between sibling spider species, small differences in courtship behavior can be enough to avoid heterospecific matings (Francescoli & Costa 1992; Töpfer-Hofmann et al. 2000; Aisenberg & Costa 2008; González et al. 2015). Finding isolation barriers mediated by differences in such characteristics may be a signature of speciation by sexual selection (Safran et al. 2013), something that may be feasible to explore in spiders.

The long-jawed orb weaver family Tetragnathidae Menge, 1866 is a highly diverse group with 988 species in 50 genera (World Spider Catalog 2021). *Tetragnatha* Latreille, 1804 species are typically abundant near aquatic environments (Costa-Schmidt et al. 2017) and often more than one species co-occur in the same site. During courtship, the male and female of some species interlock their chelicerae prior to and during copulation (Álvarez-Padilla & Hormiga 2011). In general, a remarkable cheliceral sexual dimorphism is widespread in the genus, with males having longer and stronger chelicerae than females (Álvarez-Padilla & Hormiga 2011; Baba et al. 2018; Castanheira et al. 2019).

In this paper, we explore the occurrence of heterospecific mating and evaluate whether there is a behavioral barrier that promotes reproductive isolation between two sympatric species of *Tetragnatha*: *T. argentinensis* Mello-Leitão, 1931 and *T. nitens* (Audouin, 1826). We expected that despite the similarities in morphology and general pattern of courtship behavior, the two species would display subtle differences in courtship and copulatory behaviors, and that these differences would be sufficient to avoid or limit their interbreeding. We also highlight that during our behavioral analysis, the identity of both species was initially not clear due to mismatching in the type material of *T. longidens* Mello-Leitão, 1945, which we now consider a junior synonym of *T. argentinensis* (see synonymic notes below). Additionally, a distribution map of *T. argentinensis* is provided.

METHODS

Specimen collection and housing.—We collected adult specimens of both *Tetragnatha* species in two neighboring localities separated by two kilometers from each other: Paso del Molino (34°16′40.10"S, 55°14′00.80"W) and Laguna de los Cuervos (34°16′26.6"S, 55°15′25.8′'W), Lavalleja, Uruguay. We collected 98 individuals of *Tetragnatha argentinensis* (46 females and 52 males) and 40 individuals **of** *Tetragnatha nitens* (23 females and 17 males) during January 2014, and 66 individuals **of** *Tetragnatha argentinensis* (35 females and 31 males) and 38 individuals **of** *Tetragnatha nitens* (18 females and 20 males) during December 2016. We collected mating pairs of both species in both localities to assure they were in their reproductive season. We manually captured them during the night (between 8:00 pm and 10:00 pm) and then transported them to the laboratory. We identified and sexed individuals under a dissecting stereomicroscope Olympus SZ61. We kept spiders at room temperature in plastic containers (8 cm diameter x 15 cm height). We supplied one centimeter of water inside each container and a piece of a tree branch from the same site where the spiders were collected to provide support for the spiders' webs. We fed spiders once a week with one larva of *Tenebrio molitor* Linnaeus, 1758 (Tenebrionidae; Coleoptera). We deposited voucher specimens at the arachnological collection of Laboratorio de Biología Reproductiva y Evolución, LABRE-Ar, Instituto de Diversidad y Ecología Animal (CONICET-UNC) Córdoba, Argentina and at the arachnological collection of Sección Entomología, Facultad de Ciencias, Montevideo, Uruguay.

Experimental design.—Twenty-four hours before the trials, we placed females in transparent glass cages (18.5 cm x 24.5 cm height), with two centimeters of water and a branch that provided support to build a web. The trials began when a randomly selected male was introduced into the glass cage at the top of the branch (approximately 10 cm away from the female, in the female web). In the case of mating, the trial finished when the sexual pair dislodged their chelicerae. If courtship occurred without mating, interactions were ended after 40 minutes. When courtship between individuals did not occur, trials ended after 15 minutes. To set the above-mentioned experimental time, we carried out pilot experiments with both species in laboratory conditions. When mating did not occur, the male was removed from the glass cage and a new male was introduced in the cage with the same female. We repeated this protocol with a maximum of two males. The terrarium dimensions allowed females to build webs of an effective size that permitted individuals to court and mate, and also to stay away from each other if they did not intend to interact. The mating began when the couple engaged their cheliceral fangs and ended when chelicerae were disengaged. For heterospecific mating trials, we exposed 16 females of *T. argentinensis* and 18 of *T. nitens* to heterospecific males. For intraspecific mating trials, we recorded 31 mating trials for sexual pairs of *T. argentinensis* and 18 for pairs of *T. nitens*, following the same protocol described above, using a Sony digital camera. We excluded two interactions from the analyses because the videos did not allow accurate behavioral observations.

Data recording and statistical analysis.—We analyzed the video recordings with the JWatcher software (Blumstein et al. 2000). We followed Aisenberg (2009) for the description of courtship and copulatory behaviors described for Tetragnathidae (Table 1). We estimated the number of ejaculations indirectly by counting the hematodochal expansions, as was suggested for other spiders (e.g., Aisenberg et al. 2007; González et al. 2013). We compared whether each precopulatory and copulatory behavior (see Table 1) differed between *T. argentinensis* and *T. nitens* using Markov Chain Monte Carlo General Linear Models (library MCMCglmm; Hadfield 2010), in R statistical language, version 3.0.3 (R Core Team 2016). We preferred Bayesian models over frequentist statistical models because Bayesian models deal better with the overdispersion of some variables. In each model, we included each behavior as a response variable and species as the predictor variable; no random factors were included. The distribution of error included in each model varied according to the scale of measure of each variable: Poisson for count variables (e.g., number of inflations), Gaussian for continuous variables (e.g., duration of long insertions), and categorical for binary variables (e.g., web tapping). For each model we used an inverse-Gamma prior distribution (Hadfield 2012) and ran 10,000 interactions with a burn period of 1000.

Species identification.—As with many other species of the genus, we identified the species based on the cheliceral morphology (Figs. 1–4) and genital details. We applied a variant of the phylogenetic concept of species: the diagnostic concept (Nelson & Platnick 1981; De Queiroz 2007). In a previous pilot study, we assigned the species to *T. argentinensis* and *T. longidens* Mello-Leitão, 1945, following the current

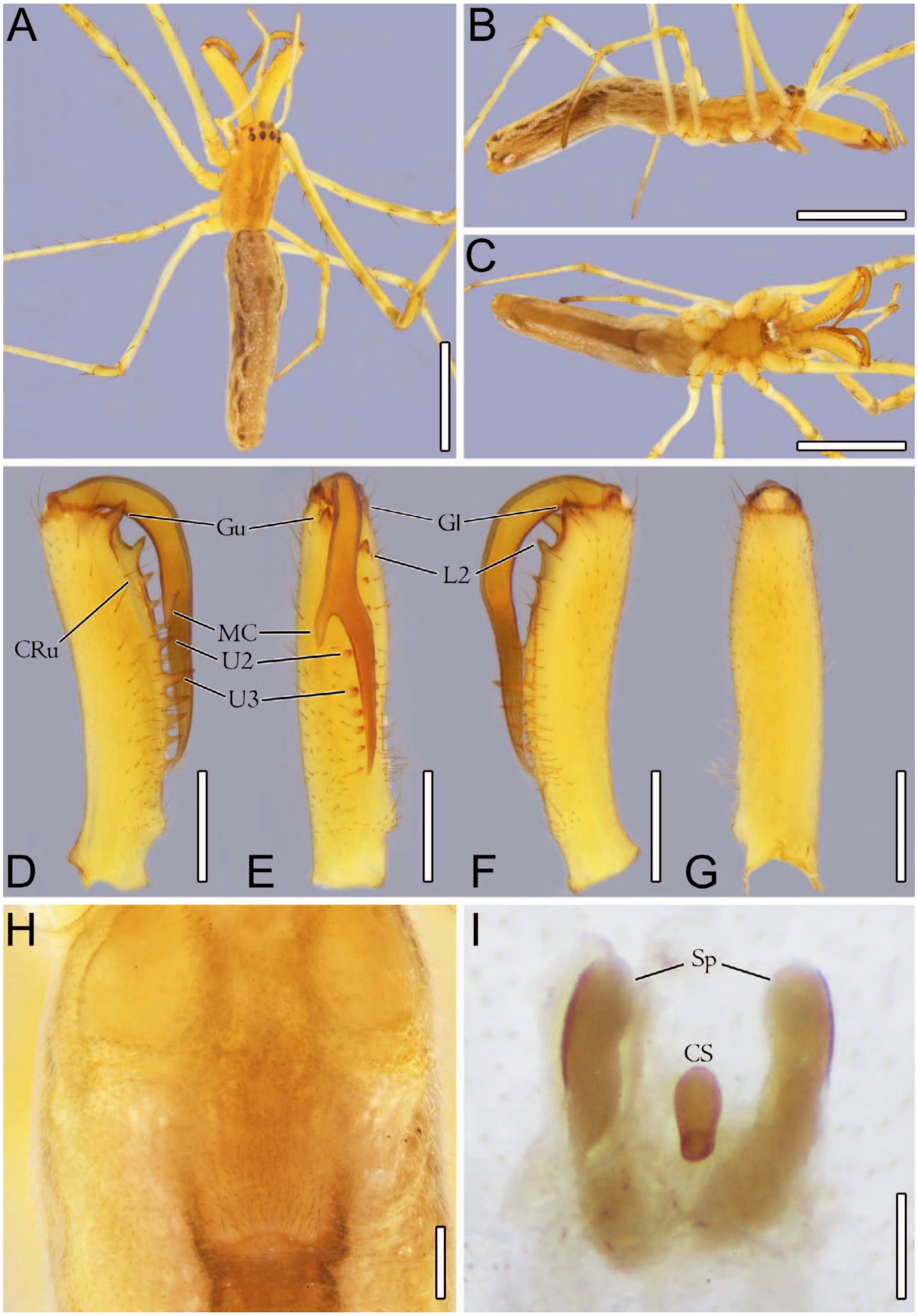

Figure 1.—*Tetragnatha argentinensis*, female (MZSP 74660 ex 5337). A–C, habitus. A, dorsal. B, lateral. C, ventral. D–G, left chelicera. D, upper view. E, inner view. F, lower view. G, outer view. H–I, female genitalia. H, genital fold, ventral view. I, internal genitalia, dorsal view (MCTP 7914). Scale bars, A, B, C, 2 mm; D, E, F, G, 0,5 mm; H, I, 0.2 mm. Abbreviations. CRu: cheliceral crest. CS: central membranous sac. Gl: guide tooth of lower row. Gu: guide tooth of upper row. L2-n: teeth on lower row (only L2 labeled). MC: median cusp. Sp: spermathecae. U2-n, teeth on upper row, numbered from distal end after Gu (only U2, U3 labeled). See Castanheira et al. (2019), Castanheira & Baptista (2020), and Castanheira & Baptista (2021a, b) for discussion of cheliceral nomenclature.

classification and original publications (World Spider Catalog 2021). However, the drawings and descriptions were not accurate enough to distinguish them from other similar species. Then we revised the type material and additional specimens deposited in scientific collections (see Supplemental File S4, online at https://doi.org/10.1636/JoA-S-21-006.s4) to confirm their taxonomic status. Besides, there was a female of the widespread and already described species *T. nitens* with the

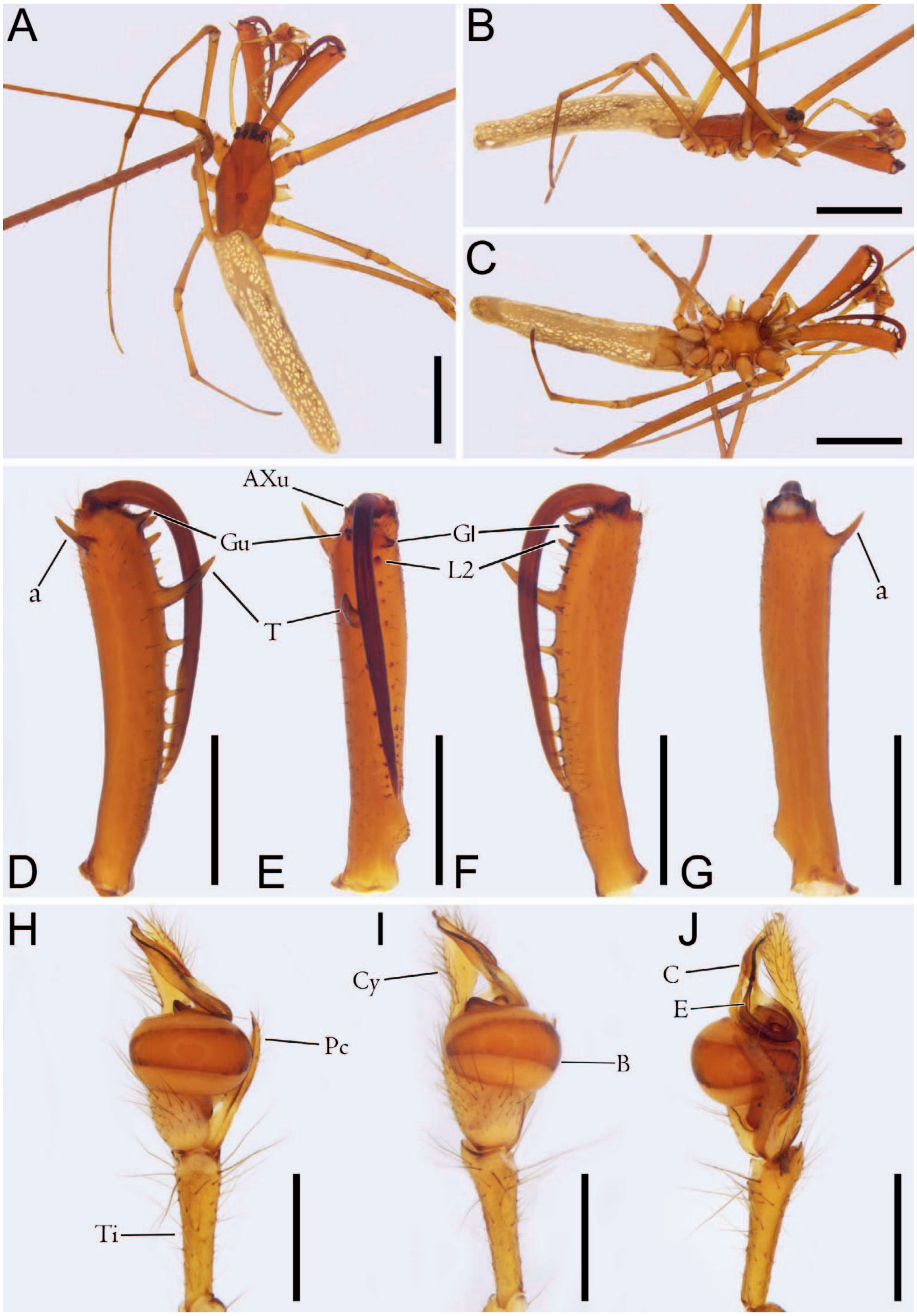

Figure 2.—*Tetragnatha argentinensis*, male (MCTP 43341 ex 13581): A–C, habitus. A, dorsal. B, lateral. C, ventral. D–G, left chelicera. D, upper view. E, inner view. F, lower view. G, outer view. H–J, left palp. H, mesal view. I, dorsal view. J, ventral view (paracymbium). Scale bars, A, B, C, 2 mm; D, E, F, G, 1 mm; H, I, J, 0.5 mm. Abbreviations. a: dorsal apophysis. AXu: auxiliary guide tooth of the upper row. B: bulb. C: conductor. Cy: cymbium. E: embolus. Gl: guide tooth of the lower row. Gu: guide tooth of upper row. L2-n: teeth of lower row (only L2 labeled). Pc: paracymbium. T: elongated tooth in the upper row. Ti: tibia. See Castanheira et al. (2019), Castanheira & Baptista (2020), and Castanheira & Baptista (2021a, b) for discussion of cheliceral nomenclature.

male type of *T. longidens*. This mismatching suggested that Mello-Leitão (1945) had not described the female of *T. longidens* (see below). We found that *T. longidens* is a junior synonym of *T. argentinensis* and that the lost male originally described as *T. argentinensis* also belonged to *T. nitens*. To be consistent throughout the text, we use the names *T. nitens* and *T. argentinensis*, dismissing *T. longidens*, proposing the formal synonymy in the taxonomic section of the results below.

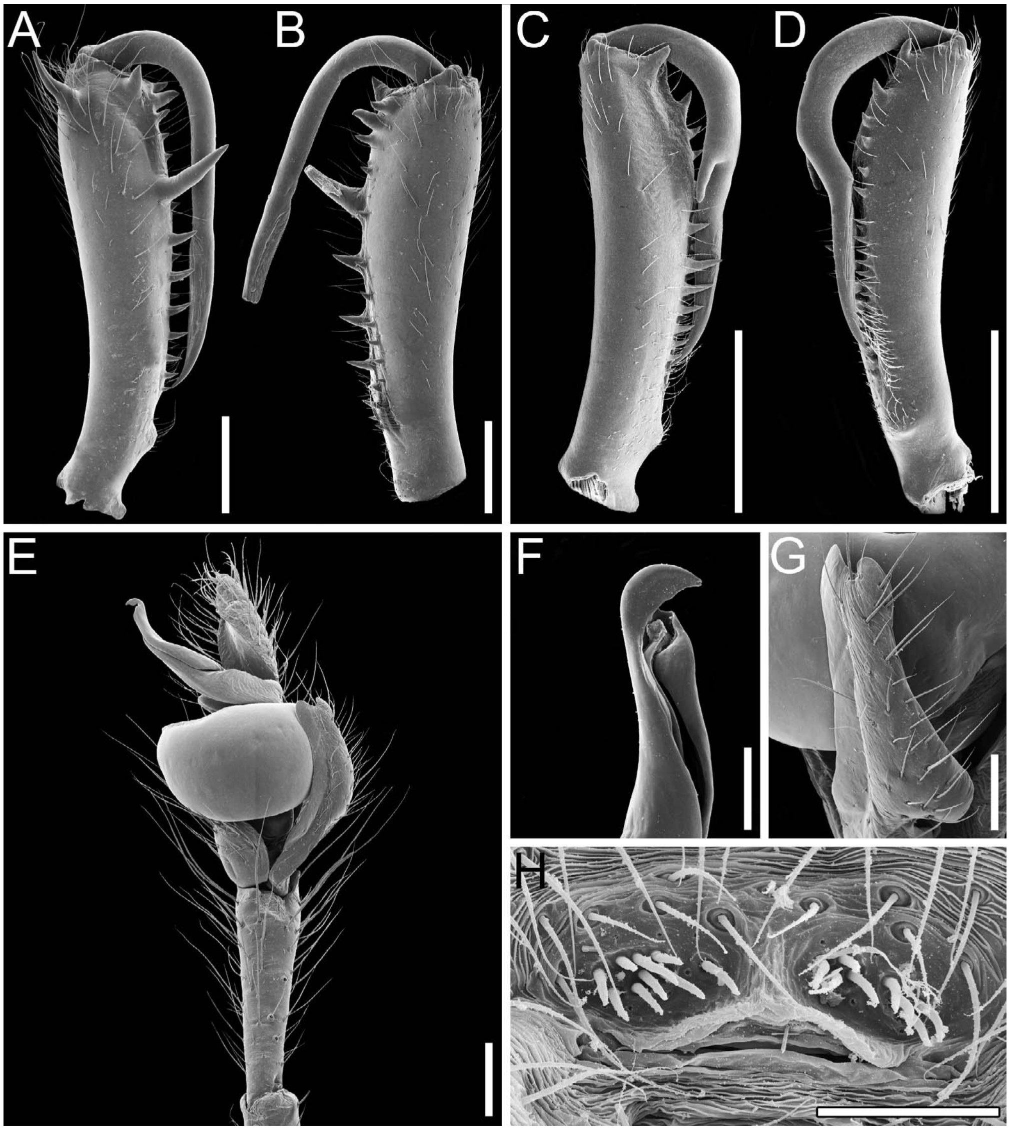

Figure 3.—*Tetragnatha argentinensis*, SEM photos: A–B, male left chelicera (MCTP 8455). A, upper view. B, lower view. C–D, female left chelicera (MCTP 41817). C, upper view. D, lower view. E–G, left male palp (MCTP 8455). E, mesal view. F, detail of condutor tip and embolus opening, dorsal view. G, paracymbium detail, ventral view. G, epiandrous detail, ventral view (MCTP 3583). Scale bars, A, B, 0.5 mm; C, D, 1 mm; E, 0.2 mm; F, H, 0.05 mm; G, 0.1 mm.

Additionally, we provide a complete list of all the material we analyzed (see Supplemental File S4). Terminology for chelicerae and other structures follows Castanheira et al. (2019, and references therein). Figures of chelicerae contain only some teeth nomenclature mentioned in the text, see Castanheira et al. (2019) for more details.

RESULTS

Heterospecific mating trials.—We recorded only one heterospecific trial among 16 interactions of a *T. argentinensis* female and *T. nitens* male. We observed pre-copulatory courtship, the male tapped the web two times, and then the pair interlocked their chelicerae following the same pattern of *T. nitens* (i.e., the males locked the females' chelicerae from the inside, using their fangs), and mating followed a similar behavioral pattern to those intraspecific matings of both species. Also, in this mating trial, there was no evidence of aggression by either sex. For comparison, we included the occurrence and the duration of the heterospecific copulation behaviors in Table 3.

During interactions that did not end in copulation, the male did not court the female, nor did the female show any apparent sign of sexual receptivity. Additionally, in these trials, we recorded three females of *T. nitens* that attacked males of *T. argentinensis*, and one female of *T. argentinensis* that attacked a male of *T. nitens*.

Intraspecific mating trials.—Both species followed a similar sequence of events during mating. The male moved on the female's web and, when the female detected the male, she advanced towards the male. Then the male approached the female and both sexes opened their chelicerae prior to clasping them. In *T. argentinensis*, females used a projection placed in

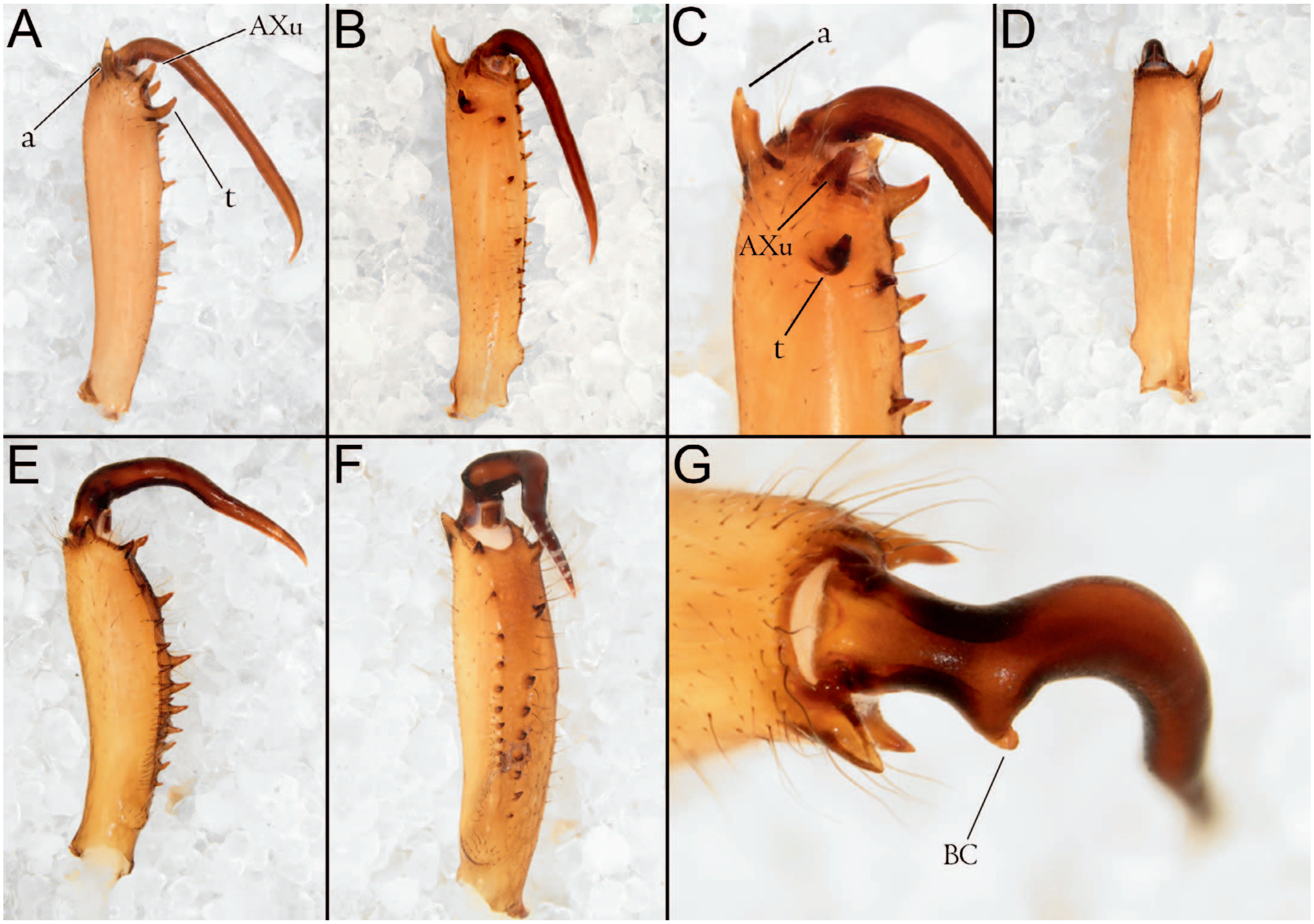

Figure 4.—*Tetragnatha nitens*, left chelicerae. A–D, male. E–G, female. A, E, upper view. B, F, inner view. C, detail of inner-anterior view. D, outer view. G, detail of apical view. Abbreviations. a: dorsal apophysis. AXu: auxiliary guide tooth of the upper row. BC: basal cusp on the cheliceral fang. t: tooth or prominence.

the middle of the fangs (MC, Fig. 1E) to internally clasp a projection in the base of males' chelicerae ('a', Figs. 2D, G). In this species, males' fangs were placed above the females' fangs. Instead, in *T. nitens*, males placed the fangs below those of females. In other words, females embraced males' chelicerae. The male used the projections of the promarginal face of the cheliceral base (a, Axu, and t, Figs. 4A, C) to clasp a projection at the base of female's fangs (BC, Fig. 4G). Once adopting the typical ventral to ventral mating position, the males touched the proximal-dorsal area of the female's abdomen with their second pair of legs, and the distal-dorsal area of the abdomen with their third pair of legs. Then, females bent the abdomen towards the males in an approximately 90° angle, enabling males to introduce part of the conductor and presumably the embolus (pedipalp insertion hereafter) into the female genital opening. During copulation males of both species used both pedipalps alternately to insert into female genitalia. Each pedipalp insertion included numerous long hematodochal expansions, presumably ejaculations. Flubs, considered failed pedipalp insertions, occurred frequently during each copulation. Females performed abdominal vibrations, identified as quick up and down movements of the abdomen. Finally, the sex that ended copulation differed according to the species. Although it is difficult to detect which sex finished the mating in our recordings due to the intense struggling, we can predict that the sex that embraces the chelicerae of its mating partner is the sex that stopped the mating. This is because the individual needs to open the chelicerae to interrupt the interlock between them. Therefore, in *T. argentinensis*, the male would be the one who finishes mating, while in *T. nitens*, it would be the female. On some occasions, we registered the females of both species chasing the males and sometimes gripping their legs with their chelicerae. We provide videos of the cheliceral interlock behavior of both species and the female abdominal bend behavior in Supplemental Videos S1-S3 (online at https://doi.org/10.1636/JoA-S-21-006.s1, https://doi.org/10.1636/JoA-S-21-006.s2, https://doi.org/10.1636/JoA-S-21-006.s3).

Sexual behaviors differences between species.—Our trials resulted in 21 copulations (out of 31 trials) for *T. argentinensis*, and 11 copulations (out of 18 trials) for *T. nitens*. We included the statistical comparisons of the precopulatory and copulatory behaviors of both species in Table 2. During the precopulatory phase, females of *T. nitens* maintained their

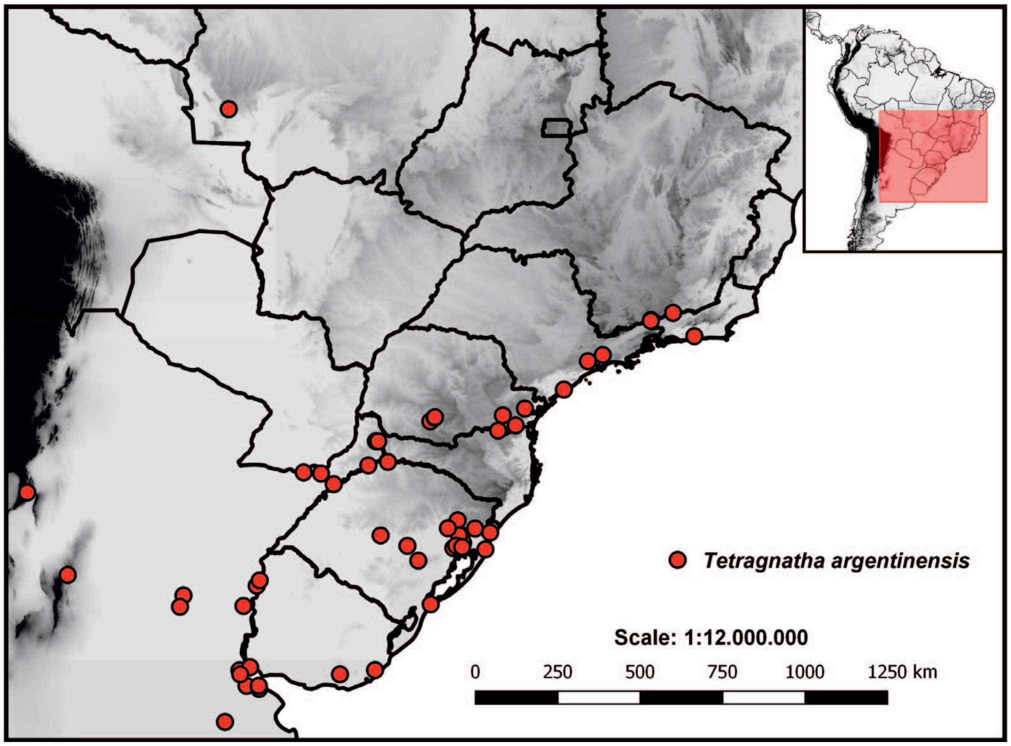

Figure 5.—Expanded distribution of *T. argentinensis*.

chelicerae opened for longer periods of time after clasping occurred, in comparison to females of *T. argentinensis* (Table 2). Other behaviors did not differ between the species. In the copulatory phase, males of *T. nitens* had fewer inflations and flubs and shorter long insertions than males of *T. argentinensis*. On the other hand, *T. argentinensis* vibrated the abdomen more often than *T. nitens* (Table 2).

Taxonomy

Family Tetragnathidae Menge 1866
Genus *Tetragnatha* Latreille, 1804
Tetragnatha argentinensis Mello-Leitão, 1931: 95, f. 1–3 (♀♂)
Tetragnatha longidens Mello-Leitão, 1945: 244, f. 22–23 (♂) **syn. nov.**
Tetragnatha soaresi Camargo, 1950: 240, pl. I, f. 3–4, pl. IV, f. 6–8 (♂).
T. l.: Camargo, 1953: 312 (syn. *T. soaresi*)
.

Type material.—*Tetragnatha argentinensis*: ARGENTINA: ♀♂, syntypes, Entre Ríos. ♀ (MNRJ 408), examined; ♂ lost.

Tetragnatha longidens: ARGENTINA: 2♂, syntypes, Misiones, Pindapoy (coll. Max Birabén, MNRJ 2307 and MLP 16425), both examined.

Table 1.—Courtship and copulatory behavioral patterns *of Tetragnatha nitens* and *Tetragnatha argentinensis* considered in the present study, with their corresponding descriptions following Aisenberg (2009).

Courtship Behavioral Patterns:	Description
Jerks the web	Facing the other sex, the individual flexes anterior legs strongly and quickly without releasing the silk line that connects them.
Taps the web	Hanging on the same line as the female, the male makes quick mesally directed taps against the line with legs I and II.
Opens chelicerae	Facing the other sex, the individuals lower their bodies and open their chelicerae.
Clasps chelicerae	Male and female interlock their cheliceral fangs.
Bends abdomen	The female bends her abdomen in a 90-degree angle assuming a ventral-to-ventral position.
Copulatory Behavioral Patterns:	
Vibrates abdomen	The female moves the abdomen quickly up and down.
Long insertions	The male inserts his pedipalp into the female genital opening and performs multiple hematodochal inflations.
Flubs	The male performs failed palpal insertion attempts.
Taps the female	The male touches or taps the female abdomen or corresponding legs with his legs I, II and III.

Table 2.—Comparison of precopulatory and copulatory behaviors in *Tetragnatha nitens* (*T. nit*) and *T. argentinensis* (*T. arg*). The intercept for each behavioral variable represents the estimate for *T. nitens*, and the second line (*T. nit -T. arg*) shows the difference between both species. Estimations of the posterior means, and confident intervals are based on the latent variables on the link scale. For comparison we included the means (Mean) and standard error (SE) estimated on our data. The value for the deviance information criterium (DIC) for each model is included in parentheses besides each response variable.

PRECOPULATORY BEHAVIORS						
Jerk the web (27.10)						
Factor	Mean	SE	Posterior mean	Confidence interval	Effective sample size	pMCMC
Intercept	1.00	0.08	−0.88	−2.34 • 0.63	530.7	0.266
T. nit -T. arg	0.71	0.10	−0.35	−2.82 • 2.08	456.9	0.800
Taps the web (22.99)						
Intercept	0.25	0.02	−2.19	−4.26 • −0.31	279.7	0.009
T. nit -T. arg	0.37	0.74	0.74	−2.31 • 3.52	284.1	0.605
Cheliceral opening duration (154.99)						
Intercept	2.18	0.38	2.20	0.01 • 4.38	1700	0.053
T. nit -T. arg	7.22	2.20	5.01	1.34 • 8.50	1700	**0.002**
Female bends abdomen (205.17)						
Intercept	11.72	0.69	11.63	6.13 • 17.72	1700	<0.001
T. nit -T. arg	18.17	2.02	6.49	−2.88 • 16.22	1484	0.173
COPULATORY BEHAVIORS						
Number of long insertions (117.66)						
Intercept	3.70	0.18	1.30	1.06 • 1.53	71.2	<0.001
T. nit -T. arg	3.22	1.98	−0.21	−0.78 • 0.18	46.6	0.366
Duration long insertions (299.77)						
Intercept	73.18	3.85	73.74	54.55 • 91.18	1832	<0.001
T. nit -T. arg	43.68	4.37	−29.32	−61.34 • 0.63	1700	0.066
Number of inflations (222.45)						
Intercept	61.16	3.21	4.06	3.85 • 4.30	1700	<0.001
T. nit -T. arg	45.60	4.56	−0.43	−0.80 • −0.04	1548	**0.027**
Number of flubs (130.35)						
Intercept	6.65	0.33	1.34	0.64 • 1.94	1465	0.001
T. nit -T. arg	2.67	0.30	−1.27	−2.50 • 0.04	1375	**0.040**
Duration of flubs (159.29)						
Intercept	11.22	0.70	11.16	6.04 • 16.13	1665	0.002
T. nit -T. arg	10.43	2.08	−7.00	−17.88 • 3.59	1700	0.186
Tap the female burst (192.46)						
Intercept	19.05	0.95	2.81	2.56 • 3.12	1700	<0.001
T. nit -T. arg	4.00	0.40	−0.25	−0.77 • 0.26	1700	0.324
Duration of tap the female burst (249.95)						
Intercept	22.20	1.17	22.10	14.56 • 30.05	1700	<0.001
T. nit -T. arg	16.56	1.65	−5.55	−18.33 • 8.44	1700	0.393
Abdomen vibration (110.77)						
Intercept	11.20	0.56	1.51	0.65 • 2.35	1398.0	0.005
T. nit -T. arg	0.10	0.01	−5.48	−8.72 • −2.57	136.2	< 0.001
Chases male (37.14)						
Intercept	0.26	0.01	−1.36	−2.26 • −0.63	61.1	<0.001
T. nit -T. arg	0.22	0.02	−0.41	−2.00 • 1.17	79.1	0.528

Table 3.—Number of occurrences and duration of courtship and copulatory behaviors of the only heterospecific mating between *T. argentinensis* female and *T. nitens* male.

	Pre-copulation				Copulation				
Behavior	Jerk the web	Tap the web	Open Chelicerae	Female Bends	Long insertion	Inflation	Flubs	Burst of tapping	Abdominal vibrations
N° of occurrences	-	2	1	1	3	80	8	6	5
Duration (sec)	-	7.507	2.647	5.378	-	136.696	12.884	-	10.377

Diagnosis.—Females of this species are morphologically similar to *T. cladogantha* Bertkau, 1880. Males, on the contrary, are more similar to *Tetragnatha elongata* Walckenaer, 1841. Females of *T. argentinensis* and *T. cladognatha* differ from all other *Tetragnatha* by the median cusp facing the upper row (MC, Fig. 1E). While females of *T. argentinensis* can be distinguished from *T. cladognatha* by a less sclerotized guide tooth of upper row ("Gu", Fig. 1D), with a wider basis, located on top of the upper crest; guide tooth of the lower row slightly more elongated ("Gl", Fig. 1F); L2 with normal narrower basis and median cusp (MC) more basally placed, without any other cusps (Figs. 1 D–G). Males of *T. argentinensis* differ from *T. elongata* by chelicerae with chelicerae dorsal apophysis pointed and not excavated ("a", Figs. 2D, E, G; Castanheira et al. 2019, figs. 5D, E, G, 7A); elongated tooth in the upper row longer and thinner (T, Figs. 2D, E; Castanheira et al. 2019, figs. 5D, E, 7A); Gu smaller and thinner, placed more distant from the fang basis and less slanted (Fig. 2D; Castanheira et al. 2019, figs. 5D, E, 7A); "sl" absent (Castanheira et al. 2019, figs. 5D, E, 7A); AXl absent (Castanheira et al. 2019, figs. 5E, F, 7A); Gl much shorter and with a shorter tip (Fig. 2F; Castanheira et al. 2019, figs. 5E, F, 7A); shorter palp tibia, of almost the same length as cymbium (Figs. 2H–J; Castanheira et al. 2019, fig. 5H); conductor tip without a tail (Figs. 2H, I, 3F; Castanheira et al. 2019, figs. 5H–J, 7C–E); paracymbium with excavated notch and wider lobe (Figs. 2J, 3G; Castanheira et al. 2019, figs 5K, 7F) and epiandrous field wider, slightly more arched, with a wider median division and more fusules (Fig. 3H; Castanheira et al. 2019, fig. 7G).

Description.—*Female:* Carapace yellow, elongated, oval, and slightly elevated on its anterior part, with four parallel dusky lines from eyes to posterior rim (Fig. 1A). Fovea yellow, with dusky borders (Fig. 1A). Labium light brown, wider than long. Sternum oval and light brown, with dusky contour (Fig. 1C). Eyes large, parallel rows, procurved, ringed in black and evenly separated, AME and PME centrally placed, separated by its length, ALE and PLE almost touching. Legs yellow, with few spines on femora (Figs. 1A, B). Chelicera paturon yellow, thick, around 4x longer than wide and about 1.4x longer than carapace, slightly curved outwards, around 30° from body median line (Fig. 1B). AXu absent (Figs. 1D–G). Upper row with seven teeth distalward projected: Gu large, pointed, almost as long as U3, with wide basis, and located on fang groove at rim of the cheliceral crest (CRu), a large, conspicuous, protruding marked area on the upper row of teeth, separated by a large gap from U2; U2 pointed and small, almost as long as U6; U3–U7 pointed and decreasing in size. AXl absent (Figs. 1D–G). Lower row with eleven teeth distalward projected: Gl short, bulky, pointed and very sclerotized, extremely projected distalward and located on fang furrow; L2 around same size as Gl, pointed, with large basis and apart from Gl by small straight gap; L3–L6 small, thin and pointed, L7–L11 small, thin and decreasing in size, L7 with almost same size as L2 but less sclerotized and L8 with almost same size as L3–L6, but with slightly larger basis. Cheliceral fang very elongated, thick, sclerotized, with pointed and large median cusp (MC) on its middle portion, facing the upper row, abruptly tapering to its tip and closing between teeth rows (Fig. 1E). Abdomen cylindrical and not enlarged or bulged, around 1.8x longer than carapace, dorsally greyish, completely covered by guanine crystals and bearing lateral black patches (Figs. 1A, B). Venter with the same color as dorsum, with median brown stripe from genital fold towards spinnerets (Fig. 1C). Genital fold slightly elevated, 2 times wider than long, thick, with parallel borders and ending in concave and wide slightly excavated tip (Fig. 1H). Internal genitalia formed by two cylindrical and rounded spermathecae, more sclerotized on the external border, and connected to subquadrate uterus externus, and a bulged and almost cylindrical sclerotized central membranous sac (CS, Fig. 1I).

Measurements.—Total length 8.82. Carapace 2.90 long, 1.24 wide. Abdomen 5.79 long, 1.74 wide. Left chelicera 2.49 long, 0.55 wide. Leg formula I–II–IV–III. Leg I: femur 8.65, patella 0.86, tibia 6.40, metatarsus 4.04 and tarsus 1.73. Leg II: patella + tibia 5.17. Leg III: patella + tibia 1.93. Leg IV: patella + tibia 4.35.

Male: Carapace, fovea, eyes, legs, legs and sternum as female, but much darker, from light brown to reddish brown (Fig. 2A). Chelicera paturon orange-brown, thin and elongated, slightly arched outwards, around 5.6x longer than wide, about as long as carapace and around 25° from body median line, tapering towards its basis (Figs. 2 A–G). 'a' very thin, pointed, elongated, distally projected and clearly slanted, located on the edge of paturon, very close to fang groove (Figs. 2D, G, 3A). AXu very reduced. 't' absent. Upper row with nine pointed and distalward projected teeth (Figs. 2D, E): Gu sclerotized and thick, with about same size as U3 and located on fang groove; 'sl' absent; 'T' very thin and extremely elongated, with wide basis, slightly projecting upward and remaining teeth on upper row after T ("rsu") with seven almost straight teeth, decreasing in size. AXl absent. Lower row with 13 teeth pointed and distalward projected teeth (Figs. 2E, F): Gl very thick and sclerotized, placed at small crest in fang groove, bearing curved tip projected downward; L2 cylindrical with narrow basis, almost adjoined to Gl; L3–L13 very small, with almost same size and uniformly spaced apart, except for the larger gap between L5 and L6. Cheliceral fang very elongated and serrated from midway, closing between teeth rows (Fig. 2E). Abdomen pale beige, but much thinner than in female, completely covered by guanine crystals and bearing scant black patches on its lateral portion (Figs. 2A–C). Epiandrous area wider than high and slightly arched, with wide median division, and bearing ten and eight fusules on each portion respectively (Fig. 3H). Palps with elongated cymbium, around same size of rounded thick tibia (Figs. 2H–J, 3E); tegulum spherical and inflated, about 1.4 wider than high (Figs. 2H, I); conductor ribbon-like, transparent, twisted from mid-way, with thick edges, completely enfolding embolus as pouch all way through, tapering towards its hook-like tip covering embolus opening as large cap (Figs. 2H–J, 3E–G); embolus very thick and sclerotized, slightly curving from midway, originating near the cymbium at middle portion of bulb and opening below conductor on a smooth curved tip (Fig. 3F); paracymbium boomerang-shaped, elongated, downward slanted, 2.9x longer than wide and tapering towards its deeply excavated notch at apex, with thin translucent lobe, medially placed, occupying little less than 50% of paracymbium length, and reaching both its basis and apex, and large elbow-like knob (Fig. 3G).

Measurements.—Total length 6.79. Carapace 2.43 long, 1.20 wide. Abdomen 4.33 long, 0.92 wide. Left chelicera 2.63 long, 0.48 wide. Leg formula I–II–IV–III. Leg I: femur 8.51, patella 1.02, tibia 7.63, metatarsus 5.89 and tarsus 2.61. Leg II: patella + tibia 5.17. Leg III: patella + tibia 1.83. Leg IV: patella + tibia 4.75.

Synonymic notes.—Mello-Leitão (1931) described *Tetragnatha argentinensis* based on specimens of both sexes from Entre Ríos province, Argentina. In the original description, Mello-Leitão indicated that the types were deposited in Buenos Aires at the MACN, but they have not been located there as stated by Galiano & Maury (1979) and the current curator. However, we examined a female deposited at MNRJ in Rio de Janeiro city (MNRJ 408), clearly identified by Mello-Leitão as *T. argentinensis* and also collected in Entre Ríos. We consider this female specimen as one of the syntypes (see notes on *T. longidens* below), but no male identified by Mello-Leitão has ever been found in MNRJ collection (Silva-Moreira et al. 2010).

Tetragnatha longidens was described by Mello-Leitão (1945) based only on one male from Pindapoy, Misiones. This male type specimen is deposited at MLP in La Plata, Argentina (MLP 16425). As remarked by Pereira et al. (1999) and later confirmed by us, in the same vial there is a female not mentioned in the original description. Again, there was a vial deposited in MNRJ, clearly marked as “typus” of *T. longidens* even though not cited in the original description. This vial (MNRJ 2308) also included an additional female specimen belonging to the same species as the female found in the MLP vial. We consider the MNRJ male specimen a syntype, as previously hinted by Silva-Moreira et al. (2010).

Unfortunately, Mello-Leitão was not consistent in the use of the word “typus” as holotype and “cotypus” as paratype, sometimes using the word “typus” meaning the whole type series (Silva-Moreira et al., 2010). As noted by Galiano & Maury (1979), Pereira et al. (1999) and Silva-Moreira et al. (2010), Mello-Leitão usually retained duplicate specimens of species he studied or described. He kept syntypes or paratypes of many species (and even some holotypes), without mentioning it in his publications. Therefore, the female of *T. argentinensis* and at least the male of *T. longidens* deposited at the MNRJ ought to be considered as syntypes.

Tetragnatha argentinensis is clearly a valid species, at least if we consider only the female syntype from MNRJ. However, the male described by Mello-Leitão (1931) seems not to be conspecific with the female, suggesting that he mismatched the specimens. Although the male syntype is lost, the precarious illustrations of its chelicera and palp indicate that it may belong to the cosmopolitan species *T. nitens*. The illustration of the male chelicera (Mello-Leitão 1931, Fig. 2) shows a large apical apophysis, the first two teeth on the upper row (AXU and t) apart from the row itself, large and of about the same size, alongside the much smaller Gu, all typical characters found in *T. nitens* (see Okuma 1983, fig. 4A; Okuma 1992, fig. 15A; Castanheira et al. 2019, figs. 14C, D, 16A). Furthermore, the illustration of the palp also does not rule out its identity as *T. nitens*. In addition, the above cited females found on the vials of *T. longidens* syntypes at MLP and MNRJ also belong to *T. nitens*, a very common species in Argentina. Fortunately, all MNRJ specimens survived the tragic 2018 fire, as they were on loan to us at that time.

The type-localities of *T. argentinensis* and *T. longidens* are compatible with the synonymy herein proposed. Both localities are placed in the Parana River basin, at northeastern Argentina, around 700 km apart, and near the border with Brazil, specifically Rio Grande do Sul state, and Uruguay. We have identified abundant males of *T. longidens* and females of *T. argentinensis* from southern Brazil, northeastern Argentina and southern Uruguay, indicating that this species is common throughout the region. In many opportunities these couples were collected together, indicating that they should belong to the same species. Taking all this into account, we established the new synonymy, *Tetragnatha longidens* Mello-Leitão, 1945 = *Tetragnatha argentinensis* Mello-Leitão, 1931 **NEW SYNONYMY!**

Distribution.—The known geographical area of this species ranges from central Brazil (Mato Grosso state) and southeastern Brazil (Minas Gerais, São Paulo and Rio de Janeiro states) to northeastern Argentina (Buenos Aires province) and southern Uruguay (Rocha province), also including Catamarca, at northwestern Argentina (Fig. 5).

DISCUSSION

This study showed certain differences at pre-copulatory and copulatory levels between *T. argentinensis* and *T. nitens*. We observed that the courtship of both species was similar to those of other species of the genus, such as *T. extensa* (Linnaeus, 1758) (West & Toft 1999), *T. elongata* Walckenaer, 1841 (Danielson-François et al. 2002), *T. versicolor* Walckenaer, 1841 (Danielson-François & Bukowski 2005) and *T. straminea* Emerton, 1884 (Simkovic & Andrade 2019). At pre-copulatory level, we found that the chelicerae interlock pattern is species-specific. We also found that females of *T. argentinensis* maintained the cheliceral opening for longer. Whether or not these behaviors are enough for sexual recognition prior to copulation is still arguable. In the heterospecific trials, the individuals showed little interaction, which may indicate that species recognition occurs before chelicerae interlock.

Only in one heterospecific trial**,** the pair interlocked their chelicerae similar to sexual pairs of *T. nitens*. In this case, the male internally clasped female chelicerae, and mating followed similar behavioral patterns to *T. argentinensis* and *T. nitens*. This would indicate that it is not a complete isolation barrier. Therefore, cheliceral clasping does not play a role in reproductive isolation, but once it occurs, it allows for an appropriate copulatory position (Baba et al. 2018; Simkovic & Andrade 2019), and/ or may enable a chelicerae stimulatory function as has been described for other tetragnathids (Eberhard 1996; Aisenberg et al. 2015; Segura-Hernández 2020; Danielson-François & Sullivan 2021). Cheliceral clasping behavior can be interpreted from a broader extreme sexual conflict perspective (Arnqvist & Rowe 2005), as a form of female or male control over copulation. Following the results, the species-specific interlock pattern suggests that males have the mating control in *T. argentinensis,* while females exert mating control in *T. nitens*. It is possible that the type of cheliceral clasping and its probable function as a mating control mechanism—in a sexual conflict framework—is in some degree related to the sex ratio in the population. This phenomenon can be observed more clearly in *T. nitens* where there is a slight bias in the population towards males (47% females, 53% males, pers. obs.). Copulation control in *T. nitens* could help the female avoid unnecessary mating. Conversely, copulation control in *T. argentinensis* is given by males. Considering this, we would have expected a sex ratio biased towards males in this species, but this did not occur. Although the sex ratio does not favor males, competition between them in the wild for access to females is common (Costa-Schmidt et al. 2017). The same has been recorded for other species of the genus *Tetragnatha* (e.g., Simkovic & Andrade 2019). Male control in *T. argentinensis* may prevent males from being displaced by rivals during copulation. It is also likely that this control allows them to maintain copulation for a longer time, which may aid in greater sperm transfer. In fact, in *T. argentinensis*, we can observe more hematodochal inflations and longer pedipalpal insertions, supporting this hypothesis. However, this hypothesis needs to be tested. Moreover, the picture is more complex and interesting than it appears since females in both species bend their abdomen to assist in pedipalp insertion, suggesting female cooperation. This behavior is common in the genus and has been analyzed from this perspective in *T. straminea* (Danielson-François et al. 2002; Simkovic & Andrade 2019). Surprisingly, in the few studies focused on tetragnathids' reproductive biology, the chelicerae interlock behavior has not been described in detail. This makes it difficult to establish a pattern within the genus although some recent studies in *T. praedonia* L. Koch, 1878 and *T. elongata* describe that the female claps the male's chelicerae from the inside, similarly to *T. argentinensis* (Baba et al. 2018; Danielson-François & Sullivan 2021). To assess how cheliceral grasping emerged as a pattern and their possible functionality, studies of fine morphology and mechanical coupling are needed.

During copulation, *T. nitens* had fewer inflations and flubs, and briefer long insertions than *T. argentinensis*, while females had a lower frequency of abdominal vibrations than *T. argentinensis*. The rate and frequency of hematodochal inflations (pedipal insertions) presumably correlate with the amount of sperm cells transferred by males (Aisenberg et al. 2015). However, in *Trichonephila clavipes* (Linnaeus, 1767), more pedipalp insertions do not correlate with more sperm suggesting that insertions may be also involved in female tactile stimulation (Linn et al. 2007, formerly *Nephila clavipes*). However, these hypotheses remain to be tested for the species included in this study. Similarly, the cause of the difference in the number of flubs between these two *Tetragnatha* species is difficult to discern since several hypotheses have been proposed for their function: failed attempts of pedipalp insertion (Watson 1991), exploratory movements, and/or male copulatory courtship (Robinson 1982; Eberhard 1996; Stratton et al. 1996; review in Eberhard & Huber 2010). The abdominal vibration also differed between females of these species, which is contrary to some species of *Leucauge* White, 1841 in which it is the male who performs such behavior (e.g., Eberhard & Huber 1998). Abdominal vibration could correlate with female sexual receptivity, which also remains to be further tested. Heterospecific mating in laboratory conditions were extremely low indicating that, despite the apparent similarities in these *Tetragnatha* species, there may be at least behavioral and chemical signaling barriers (e.g., different pheromone profiles), rather than ecological barriers (e.g., preference for different microhabitats) that limit the heterospecific interbreeding. Several studies have found a pheromonal divergence in closely-related species in sympatry to avoid hybridization (review in Symonds & Elgar 2008). In nature, *T. argentinensis* and *T. nitens* construct webs in the same microhabitat. Their activity and reproduction overlap, suggesting that there are no ecological barriers that could limit the inter-specific encounters between males and females of both species. Still, we cannot rule out that there are other factors to explore in a complex habitat like riverbanks, for example, plant species or other characteristics of the sites where they build their webs. There is no information on the sympatric history of these species. *Tetragnatha nitens* is a cosmopolitan species introduced in the Americas, first cited in Peru in 1878 (Taczanowski 1878). Thus, if we consider that the species was already present in America around 140 years ago, the sympatry of these species is relatively recent. Furthermore, phylogenetic information is insufficient to determine how closely related the two species may be and the divergence times of the possible clades they belong to. Therefore, at least some differences found between the species may have evolved prior to their geographic contact in South America. Still, others could have evolved or magnified their differences to reduce heterospecific mating (Mabry & Verrell 2004). This could be tested by performing heterospecific mating with individuals from non-overlapping distributions, predicting, in such case, a lower selection against interbreeding (see Mabry & Verrell 2004)

In conclusion, our results show the occurrence of reproductive isolation between both species of *Tetragnatha*. We found behavioral differences at the precopulatory and copulatory levels. These differences may have not exclusively evolved in the context of reproductive isolation since heterospecific sexual encounters were extremely rare, even in laboratory conditions. It is then likely that species-specific pheromonal profiles may play an important role in recognition in these species.

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SUPPLEMENTAL MATERIALS

Supplemental video S1.—*Tetragnatha nitens*, cheliceral interlock behavior. Online at https://doi.org/10.1636/JoA-S-21-006.s1

Supplemental video S2.—*Tetragnatha argentinensis*, cheliceral interlock behavior. Online at https://doi.org/10.1636/JoA-S-21-006.s2

Supplemental video S3.—*Tetragnatha argentinensis,* female abdominal bending behavior. The abdominal bending behavior is similar in both *Tetragnatha* species. Online at https://doi.org/10.1636/JoA-S-21-006.s3

Supplemental file S4.—Depository Institutions and Material Examined. Online at https://doi.org/10.1636/JoA-S-21-006.s4

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