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High and dry: integrative taxonomy of the Andean spider genus *Nerudia* (Araneae: Pholcidae)

BERNHARD A. HUBER[1,*], GUANLIANG MENG[1], JIŘÍ KRÁL[2], IVALÚ M. ÁVILA HERRERA[2], MATIAS A. IZQUIERDO[3,4] and LEONARDO S. CARVALHO[5]

[1]*Zoological Research Museum Alexander Koenig, LIB, Bonn, Germany*
[2]*Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic*
[3]*Facultad de Ciencias Exactas, Físicas y Naturales, Departamento de Diversidad Biológica y Ecología, Universidad Nacional de Córdoba, Córdoba, Argentina*
[4]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Laboratorio de Biología Reproductiva y Evolución, Instituto de Diversidad y Ecología Animal (IDEA), Córdoba, Argentina*
[5]*Campus Amílcar Ferreira Sobral, Universidade Federal do Piauí, Floriano, Brazil*

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Ninetinae are a group of poorly known spiders that do not fit the image of 'daddy long-legs spiders' (Pholcidae), the family to which they belong. They are mostly short-legged, tiny and live in arid environments. The previously monotypic Andean genus *Nerudia* exemplifies our poor knowledge of Ninetinae: only seven adult specimens from two localities in Chile and Argentina have been reported in the literature. We found representatives of *Nerudia* at 24 of 52 localities visited in 2019, mostly under rocks in arid habitats, up to 4450 m a.s.l., the highest known record for Pholcidae. With now more than 400 adult specimens, we revise the genus, describing ten new species based on morphology (including SEM) and *COI* barcodes. We present the first karyotype data for *Nerudia* and for its putative sister-genus *Gertschiola*. These two southern South American genera share a $X_1X_2X_3Y$ sex chromosome system. We model the distribution of *Nerudia*, showing that the genus is expected to occur in the Atacama biogeographic province (no record so far) and that its environmental niche is phylogenetically conserved. This is the first comprehensive revision of any Ninetinae genus. It suggests that focused collecting may uncover a considerable diversity of these enigmatic spiders.

ADDITIONAL KEYWORDS: Argentina – barcodes – biogeography – distribution modelling – *Gertschiola* – highest record – new species – NOR – sampling bias – sex chromosomes.

INTRODUCTION

Pholcid spiders are among the most species-rich spider families (World Spider Catalog, 2022), and highly diverse regarding the microhabitats they occupy and their corresponding body shapes and colours (Eberle *et al.*, 2018). They are well known to many people by a few synanthropic species. However, the mainly tropical and subtropical distribution of this group has long caused a fragmentary knowledge about the family as a whole. Intensive research over the last two decades has not only resulted in the discovery and description of more than 1000 new species, but has also revealed some facts about their biology. For example, multiple convergent shifts among microhabitats have resulted in a wide range of ecomorphs, with strong convergencies in body shape, coloration and behaviour (Dimitrov *et al.*, 2013; Eberle *et al.*, 2018). Sexual dimorphisms have evolved over 100 times independently (Huber, 2021), including some spectacular phenomena like males with extremely modified 'head' structures (Huber & Nuñeza, 2015; Huber *et al.*, 2016). Females guard their eggs by carrying the egg-sacs in their chelicerae, but parasitic wasps have repeatedly managed to access the eggs

*Corresponding author. E-mail: b.huber@leibniz-lib.de
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and use the spider female to protect the developing offspring of her enemy (Huber & Wunderlich, 2006; Johnson *et al.*, 2018). In keeping with their species diversity, pholcids exhibit a considerable diversity of diploid numbers, sex chromosome systems and patterns of nucleolus organizer regions (NORs) (Ávila Herrera *et al.*, 2021).

The family is currently divided into five subfamilies (Huber, 2011). Among these, Ninetinae are of particular interest in many respects. When Eugène Simon constructed the first classification of Pholcidae (Simon, 1893), he placed the single Ninetinae species known to him in a separate subfamily, opposing all other pholcids. While molecular data have not yet resolved the position of Ninetinae (Eberle *et al.*, 2018), sperm data support a sister-group relationship between Ninetinae and all other Pholcidae: most Ninetinae have synspermia (Dederichs *et al.*, 2022), a sperm transfer form that characterizes ecribellate Haplogynae spiders (= Synspermiata *sensu* Michalik & Ramírez, 2014). This contrasts with all other pholcids studied, which have cleistospermia. Apart from their possible 'basal' position among Pholcidae, Ninetinae are 'unusual' in several respects: (1) Ninetinae are largely restricted to arid habitats, while most other pholcids (in particular those in the New World) are concentrated in humid tropical environments (e.g. Huber & Brescovit, 2003); (2) while Ninetinae contain only 2% of extant Pholcidae species, they contain 20% (three of 15) of the known fossil species (Dunlop *et al.*, 2020); (3) with previously only 32 (now 42) described species, Ninetinae are by far the smallest of the five subfamilies in Pholcidae; (4) Ninetinae have by far the lowest mean number of species per genus (previously 2.5, now 3.2 – other subfamilies: 11.2–27.6), suggesting old diversification with little subsequent speciation or displacement by modern taxa; and (5) Ninetinae are small to tiny (body length usually 1–2 mm; most other pholcids range from 2 to 10 mm) and their often short legs do not fit the image of a 'daddy long-legs spider'.

Almost nothing has been known about Ninetinae biology beyond basic data on microhabitat, i.e. on reproductive biology, web building, predatory behaviour or life cycle. Label data and preliminary field observations have indicated that Ninetinae are fast-running ground spiders and that at least some species may not even build webs. This is remarkable considering that among the closest relatives of Pholcidae [phylogeny of Wheeler *et al.* (2017)], some taxa make webs (e.g. Diguetidae), while others apparently do not (e.g. Tetrablemmidae).

Geographically, Ninetinae seem to be relatively diverse in the New World (12 named genera), while only two genera are currently described from the Old World. Brazil has the highest number of genera (four named), followed by Argentina and Venezuela (three named genera each). In Argentina and Canada, representatives of Ninetinae mark the most southern and most northern pholcid records worldwide (excluding synanthropic species; –47.7° and 56.1°; M. Ramírez, pers. comm. Jan. 2022; Bennett, 2014). To this we add here the highest record, supporting the idea that Ninetinae tolerate more extreme environmental conditions than other Pholcidae.

The genus *Nerudia* Huber, 2000 has long been a typical example of our limited knowledge of Ninetinae. It was described in 2000 for a single Chilean species (*Nerudia atacama* Huber, 2000), based on five adult specimens from two neighbouring localities (Huber, 2000). Since then, only two further adult specimens of *Nerudia* have been reported in the literature (Torres *et al.*, 2015). Based on morphology, they were (erroneously) assigned to *N. atacama*, even though they originated from Salta Province in Argentina, 700 km NE of the type locality.

In 2019, two of us (BAH, MAI) conducted an expedition in Argentina, aiming primarily at Ninetinae. To our surprise, *Nerudia* spiders were found at 24 of the 52 visited localities, often in considerable abundance. The present paper describes this material, raising the number of nominal species from one to 11. We provide the first karyotype data of *Nerudia* and of the putative sister-genus *Gertschiola* Brignoli, 1981. Finally, we study the biogeography of *Nerudia* in an effort to estimate the potential distribution of the genus, to test the environmental niche conservatism in the group and possible biases in the available locality data, which are likely to result in biodiversity shortfalls regarding this genus. In particular, our biogeographic analyses aim to test two hypotheses: (1) among Ninetinae, *Nerudia* representatives occupy a unique environmental niche; and (2) the modelled environmental niche of *Nerudia* suffers from sampling biases.

MATERIAL AND METHODS

Taxonomy and morphology

The taxonomic part of this study is based on the examination of 410 adult specimens deposited in the following collections: American Museum of Natural History, New York, USA (AMNH); Laboratorio de Biología Reproductiva y Evolución, Córdoba, Argentina (LABRE); Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina (MACN); and Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK).

Taxonomic descriptions follow the style of recent publications on Pholcidae (e.g. Huber, 2022; based on: Huber, 2000). Measurements were done on a dissecting microscope with an ocular grid and are in mm unless

otherwise noted; eye measurements are +/– 5 µm. Live specimens (Fig. 1) were photographed with a Canon EOS 700D camera. Other photos were made with a Nikon Coolpix 995 digital camera (2048 × 1536 pixels) mounted on a Nikon SMZ 18 stereo microscope or a Leitz Dialux 20 compound microscope. COMBINEZP (https://combinezp.software.informer.com/) was used for stacking photos. Drawings are partly based on photos that were traced on a light table and later improved under a dissecting microscope, or they were directly drawn with a Leitz Dialux 20 compound microscope using a drawing tube. Cleared epigyna were stained with chlorazol black. The number of decimals in coordinates gives a rough indication about the accuracy of the locality data: four decimals means that the collecting site was within about 10 m of the indicated spot; three decimals: within ~100 m; two decimals: within ~1 km; one decimal: within ~10 km. Distribution maps were generated with ARCMAP v.10.0 (Environmental Systems Research Institute, Redlands, CA). For scanning electron microscope (SEM) photos, specimens were dried in hexamethyldisilazane (HMDS) (Brown, 1993) and photographed with a Zeiss Sigma 300 VP scanning electron microscope. SEM data are presented with the descriptions but are usually not based on the specific specimen described. Abbreviations used in figures only are explained in the figure legends. Abbreviations used in the text: ALE, anterior lateral eye(s); ALS, anterior lateral spinneret(s); AME, anterior median eye(s); a.s.l., above sea level; L/d, length/diameter; PME, posterior median eye(s); PMS, posterior median spinneret(s).

MOLECULAR DATA AND ANALYSES

Taxon sampling

Outgroup taxa were selected from Eberle *et al.* (2018), including ten Ninetinae species and two *Artema* species (Pholcidae: Arteminae) for rooting the tree. Additionally, we newly sequenced 24 *Nerudia* and nine *Gertschiola* specimens. In total, the molecular study includes 47 specimens. Table 1 lists the newly sequenced specimens. Accession numbers of previously sequenced specimens are shown in the Supporting Information (Figs S1, S2); for detailed information on these specimens, see Eberle *et al.* (2018). Two to four legs of specimens stored in pure ethanol (~99%) at –20 °C were used for DNA extraction. Extracted genomic DNA is deposited at and is available from the LIB Biobank, Museum Koenig, Bonn.

DNA extraction, amplification and sequencing

For 32 specimens (24 *Nerudia* specimens and eight *Gertschiola* specimens; Table 1), DNA was extracted using the HotSHOT method (Truett *et al.*, 2000). The primer set used was LCO1490-JJ and HCO2198-JJ (Astrin *et al.*, 2016; primer versions JJ2 served as backup), with different tag sequences (from Srivathsan *et al.*, 2021) of 13 bp length added at the 5′-ends of forward and reverse primers, respectively. The 20 µL reaction volume consisted of 5 µL H_2O, 2 µL Q-Solution, 10 µL Qiagen Multiplex-Mix, 1 µL forward primer, 1 µL reverse primer and 1 µL DNA. The polymerase chain reaction (PCR) procedure was: (1) 95 °C for 15 min; (2) denaturation at 94 °C for 35 s; (3) annealing at 55 °C (or 40 °C) for 90 s; (4) elongation at 72 °C for 90 s; and (5) final elongation at 72 °C for 10 min, followed by cooling at 10 °C. Steps 2–4 were repeated for 15 cycles (or 25 cycles). The PCR products (each with a different combination of 13 bp tags) were then pooled and sequenced with the Oxford Nanopore Technologies (ONT) GridION platform, making use of the SQK-LSK110 sequencing kit and a flongle R9.4 flowcell. One *Gertschiola* specimen (N010) was amplified with the same primer set but its PCR product was sent for bidirectional Sanger sequencing to BGI (Hong Kong).

Cytochrome c oxidase subunit I (COI) *barcode assembly and contamination check*

A total of 32 *COI* barcodes were sequenced by ONT sequencing and assembled following the ONTbarcoder (Srivathsan *et al.*, 2021) pipeline, while one *COI* barcode was characterized with Sanger-sequencing and assembled and aligned with GENEIOUS R7 (Kearse *et al.*, 2012). To confirm the taxonomic assignments and to identify contaminations, the assembled sequences were checked by: (1) blasting against a local NT database and (2) the identification engine of the Barcode of Life Data System (BOLD) (http://www.boldsystems.org/index.php) (Ratnasingham & Hebert, 2007; Yang *et al.*, 2020).

Multiple sequence alignment (MSA) and neighbour-joining (NJ) tree construction

A total of 47 *COI* barcodes were translated into protein sequences using BIOPYTHON v.1.78 (Cock *et al.*, 2009) and the option for the invertebrate mitochondrial genetic code. Protein-MSAs were constructed using the mafft-linsi algorithm of MAFFT v.7.299b (Katoh & Standley, 2013), which then guided the construction of nucleotide level MSAs with pal2nal.pl (Suyama *et al.*, 2006). With this procedure we avoided the introduction of biologically meaningless frameshifts to the alignments (Suyama *et al.*, 2006). Finally, MEGA X (Kumar *et al.*, 2018; Stecher *et al.*, 2020) was used for the construction of a NJ tree (Saitou & Nei, 1987) based on the nucleotide alignment. Evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and all ambiguous positions were removed for each sequence pair (pairwise deletion

Table 1. Geographic origins of newly sequenced specimens, sorted by code, and their *COI* GenBank accession numbers

Code	Genus	Species	Vial	Country	Admin.	Locality	Lat	Long	*COI*
N001	*Nerudia*	*nono*	Arg124	Argentina	Córdoba	~5 km E Nono	-31.7982	-64.9515	ON063450
N004	*Gertschiola*	*macrostyla*	Arg128	Argentina	Córdoba	between Villa Dolores and Chancani	-31.8328	-65.2647	ON067457
N006	*Gertschiola*	*macrostyla*	Arg134	Argentina	San Juan	Cuesta de Marayes	-31.4949	-67.3358	ON067458
N007	*Nerudia*	*ola*	Arg135	Argentina	San Juan	~7.5 km S Astica	-31.0223	-67.2976	ON063451
N008	*Nerudia*	*ola*	Arg136	Argentina	San Juan	~7.5 km S Astica	-31.0223	-67.2976	ON063452
N010	*Gertschiola*	*macrostyla*	Arg140	Argentina	San Juan	San Agustín de Valle Fértil	-30.6366	-67.4863	ON062532
N011	*Nerudia*	*ola*	Arg141	Argentina	San Juan	San Agustín de Valle Fértil	-30.6366	-67.4863	ON063453
N012	*Nerudia*	*ola*	Arg144	Argentina	San Juan	Parque Provincial Ischigualasto	-30.1839	-67.9026	ON063454
N013	*Gertschiola*	*macrostyla*	Arg145	Argentina	San Juan	Parque Provincial Ischigualasto	-30.1839	-67.9026	ON067459
N014	*Nerudia*	Arg23a	Arg147	Argentina	San Juan	~35 km W Las Flores	-30.3967	-69.5576	ON063455
N015	*Nerudia*	*rocio*	Arg148	Argentina	San Juan	~35 km W Las Flores	-30.3967	-69.5576	ON063456
N016	*Gertschiola*	*macrostyla*	Arg149	Argentina	San Juan	between San José de Jáchal and Huaco	-30.1497	-68.6063	ON067460
N017	*Nerudia*	*ola?*	Arg152	Argentina	La Rioja	Cuesta de Miranda, 'site 1'	-29.3511	-67.7924	ON063457
N018	*Nerudia*	*ola?*	Arg154	Argentina	La Rioja	Cuesta de Miranda, 'site 2'	-29.3468	-67.7205	ON063458
N019	*Gertschiola*	sp.	Arg158	Argentina	La Rioja	between Chilecito and Famatina	-29.0027	-67.4855	ON067461
N020	*Nerudia*	*hoguera*	Arg159	Argentina	La Rioja	between Chilecito and Famatina	-29.0027	-67.4855	ON063459
N021	*Nerudia*	*ola?*	Arg161	Argentina	La Rioja	SE Aimogasta, 'site 1'	-28.8069	-66.6635	ON063460
N023	*Nerudia*	Arg163	Arg163	Argentina	La Rioja	SE Aimogasta, 'site 2'	-28.9015	-66.6538	ON063461
N024	*Gertschiola*	sp.	Arg164	Argentina	La Rioja	SE Aimogasta, 'site 2'	-28.9015	-66.6538	ON067462
N025	*Nerudia*	*guirnalda*	Arg166	Argentina	Catamarca	El Rodeo, trail to Cristo Redentor	-28.2229	-65.8677	ON063462
N026	*Nerudia*	*guirnalda*	Arg167	Argentina	Catamarca	El Rodeo, trail to Cristo Redentor	-28.2229	-65.8677	ON063463
N028	*Nerudia*	*colina*	Arg178	Argentina	Jujuy	betw. San Salvador and Purmamarca, 'site 2'	-23.8849	-65.4613	ON063464
N030	*Nerudia*	*poma*	Arg184	Argentina	Salta	~15 km NW Campo Quijano	-24.7918	-65.7297	ON063465
N033	*Nerudia*	*poma*	Arg195	Argentina	Salta	Cabra Corral, 'site 3'	-25.2907	-65.3057	ON063466
N035	*Nerudia*	*poma*	Arg197	Argentina	Salta	Cabra Corral, 'site 4'	-25.2837	-65.3939	ON063467
N036	*Nerudia*	*trigo*	Arg202	Argentina	Salta	~1 km SW Alemanía	-25.6300	-65.6180	ON063468
N038	*Nerudia*	*trigo*	Arg206	Argentina	Salta	between Alemanía and Cafayate	-25.7023	-65.7022	ON063469
N040	*Nerudia*	*ola?*	Arg211	Argentina	Catamarca	near Nacimientos	-27.1559	-66.6925	ON063470
N041	*Nerudia*	*ola?*	Arg213	Argentina	Catamarca	~10 km N Belén	-27.5641	-67.0058	ON063471
N042	*Gertschiola*	sp.	Arg214	Argentina	Catamarca	~14 km W Fiambalá	-27.6590	-67.7607	ON063472
N043	*Nerudia*	*centaura*	Arg215	Argentina	Catamarca	~20 km E Paso de San Francisco, 'site 1'	-26.9276	-68.0709	ON063473
N044	*Nerudia*	*centaura*	Arg216	Argentina	Catamarca	~20 km E Paso de San Francisco, 'site 2'	-26.9360	-68.0925	ON063474
N056	*Gertschiola*	*macrostyla*	MACN267	Argentina	San Luis	PN Sierra de las Quijadas	-32.4690	-66.9609	ON067463

option). Branch supports were evaluated with 1000 bootstrap replicates (Felsenstein, 1985). The NJ tree in Figure 2 shows only the ingroup taxa. The complete tree is shown in the Supporting Information (Figure S1).

Preparation of chromosome slides and their evaluation

Chromosomes were prepared from two specimens of the new species *Nerudia poma*, two specimens of the new species *Nerudia ola* and one specimen of *Gertschiola macrostyla* (Mello-Leitão, 1941). Chromosome plates were obtained from testes of adult males. In both species of *Nerudia*, the preparations contained both mitotic and meiotic plates. In *G. macrostyla*, they only contained meiotic plates. Chromosome preparation was based on Dolejš *et al.* (2011). Tissues were hypotonized in 0.075M KCl for 25 min at room temperature (RT) and fixed two times (10 and 20 min) in ethanol : acetic acid (3 : 1) (RT). To make a cell suspension, a piece of fixed tissue was placed on a microscope slide with a drop of 60% acetic acid and quickly shredded with a pair of tungsten needles to obtain a cell suspension. The preparation was placed on a histological plate (40 °C). The drop was

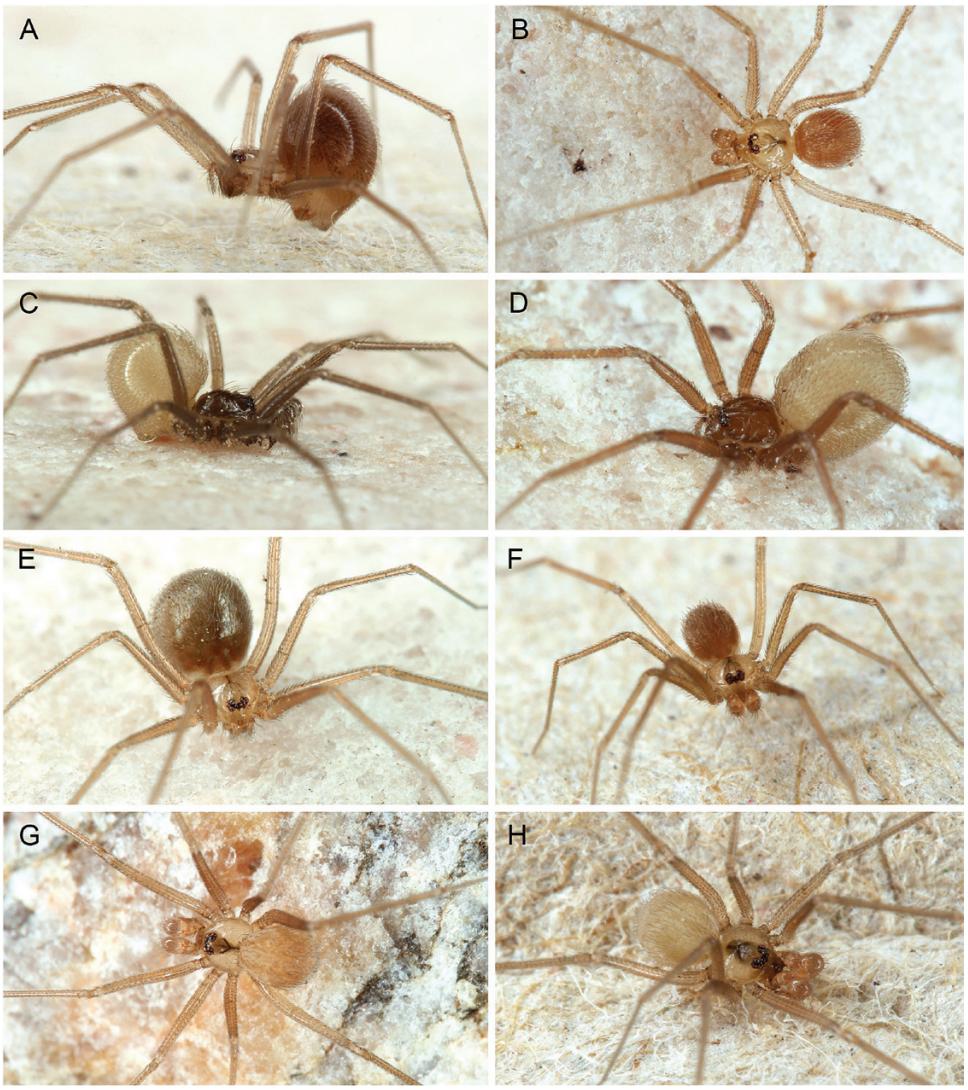

Figure 1. *Nerudia*, live specimens from Argentina. A, *N. colina* sp. nov., female from Jujuy, between San Salvador and Purmamarca (type locality). B, *N. poma* sp. nov., male from Salta, NW Campo Quijano (type locality). C, D, *N. centaura* sp. nov., male and female from Catamarca, E Paso de San Francisco (type locality). E, *N. trigo* sp. nov., female from Salta, SW Alemania. F, *N. guirnalda* sp. nov., male from Catamarca, El Rodeo (type locality). G, *N. ola* sp. nov., male from San Juan, San Agustin de Valle Fertil (type locality). H, *N. nono* sp. nov., male from Cordoba, E Nono (type locality).

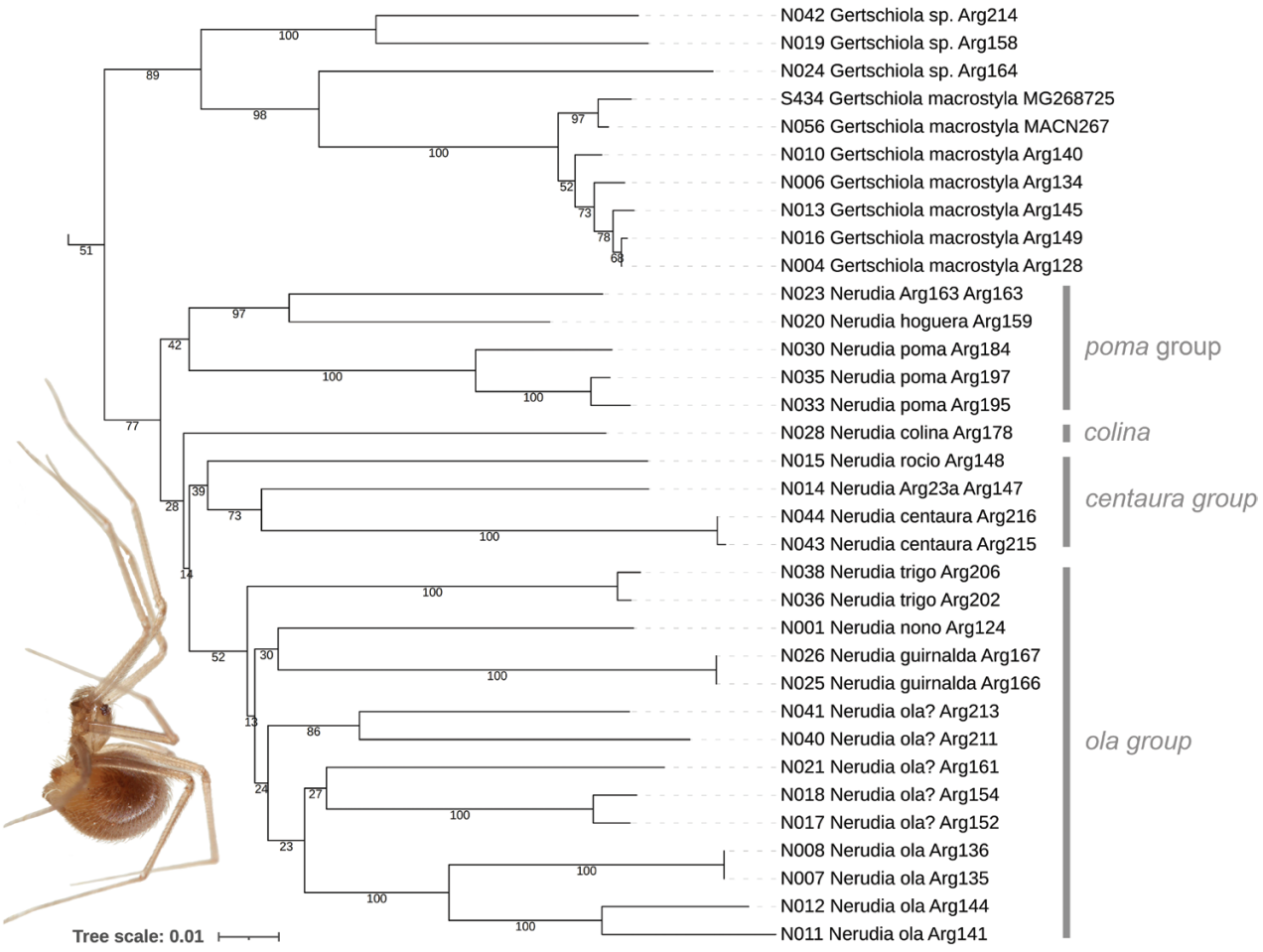

Figure 2. NJ *COI* tree of all sequenced *Nerudia* and *Gertschiola* specimens. Species groups on the right are working hypotheses. Photo: female of *Nerudia colina* sp. nov. For complete tree, and for tree resulting from analysis with IQ-TREE, see Supporting Information, Figures S1, S2.

moved with a tungsten needle until it almost evaporated. The remaining suspension was discarded. Slides were stained with a 5% Giemsa solution in Sörensen buffer (pH 6.8) for 28 min (RT).

Preparations were studied under an Olympus BX 50 microscope equipped with a DP 71 CCD camera. Chromosome morphology was characterized based on the position of the centromere (Levan, 1964), which was calculated as the ratio of the longer and shorter chromosome arms. Chromosomes were measured using the IMAGE TOOL v.3.0 software (https://imagetool.software.informer.com/3.0/). Metacentric and submetacentric chromosomes were considered as biarmed, and subtelocentric and acrocentric chromosomes as monoarmed. The sex chromosome system was identified from meiosis of the heterogametic sex, based on segregation of the sex chromosomes and/or their behaviour in prophase and metaphase I.

VISUALIZATION OF NUCLEOLUS ORGANIZER REGIONS (NORS)

After inspection under the Olympus BX 50 microscope, the slides were treated in xylene and benzine baths (1 min each, RT) to remove the immersion oil. The Giemsa stain was removed from preparations by incubation in the fixative (3 min, RT). Nucleolus organizer regions were detected by a biotin labelled 18S rDNA probe from the spider *Dysdera erythrina* (Walckenaer, 1802) (Dysderidae) (for details, see: Ávila Herrera *et al.*, 2021). The probe was visualized by fluorescence *in situ* hybridization (FISH) using streptavidin-Cy3 with signal amplification (biotinylated antistreptavidin, streptavidin-Cy3). Chromosomes were counterstained by DAPI (for description of method, see: Forman *et al.*, 2013). Chromosome plates were captured with an Olympus IX81 microscope equipped with an ORCA-AG CCD camera (Hamamatsu). Images were pseudocoloured (red for Cy3, blue for DAPI) and

superimposed with the CELL^R software (Olympus Soft Imaging Solutions).

SAMPLING BIAS AND BIOGEOGRAPHIC ANALYSES

In order to test if the environmental niche occupied by *Nerudia* representatives differs from other ninetines and if it suffers from sampling biases, the numbers of records of arthropods and arachnids in the wider geographic area of *Nerudia* were used as predictors in generalized linear models with a binomial distribution of errors. To perform these analyses, a database with records of preserved specimens of arthropods from Argentina, Chile, Bolivia and Paraguay was gathered from the Global Biodiversity Information Facility (GBIF.org, 2022). The GBIF search was restricted to records with true coordinates and without known geospatial issues detected by the platform. It yielded 404 541 records of arthropods, including 36 796 records of arachnids. These records were further spatially clipped to a 0.5-degree side-by-side hexagon grid, built at a 500-km radius from all *Nerudia* records. This resulted in a database with 131 955 records of arthropods, including 9032 records of arachnids. A kernel density map was calculated to provide an overview of the sampling effort in the study area. This analysis was performed at ARCGIS v.10.3, with an output cell size of 0.02, search radius of one decimal degree and a planar method. All raster files were plotted using stretched symbology of 2.5 standard deviations.

As a qualitative exploratory analysis, areas with high suitability for representatives of *Nerudia* (i.e. areas with higher indices resulting from species distribution modelling predictions) were modelled using all *Nerudia* species records. No *Nerudia* species has enough known records to allow individual modelling. Thus, we disregarded individual species environmental thresholds and treated them as a single biological entity in model inputs. As predictors, we used the 19 climatic variables available in the WorldClim v.2 database (https://www.worldclim.org/data/index.html), plus the mean tree density (Crowther *et al.*, 2015) and mean canopy height (Simard *et al.*, 2011), both at a 1-km^2 scale. These variables were selected to provide a satisfactory description of biological and climatic conditions of the habitats of Pholcidae species, which occupy a large variety of environments worldwide. To remove the spatial autocorrelation between the predictor layers, a principal component analysis (PCA) was carried out in R v.4.1.0 (R Development Core Team), using tools in the packages 'raster' (Hijmans *et al.*, 2016), 'rgdal' (Bivand *et al.*, 2019) and 'RStoolbox' (Leutner *et al.*, 2019). The principal components that summed at least 95% of the proportion of variance were used as predictors in the modelling (Supporting Information, Tables S1, S2). The modelling was performed with MAXENT, without applying a threshold rule, with 500 maximum interactions, random test percentage of 25%, raw output formatted, with 15 bootstrap replicates and by removing duplicates from the same grid cell (following the recommendations by: Merow *et al.*, 2013). The map representing the median of the replicates is shown in Figure 43B, and the contribution of the principal components to the species distribution modelling is in Table S3. In the sections on biogeography, we adopted the biogeographic regionalization of the Neotropical region and its hierarchy (i.e. regions, dominions, provinces and districts) proposed by Morrone (2017). The terms used here should not be mistaken for political administrative areas.

The same environmental predictors were used to test if the environmental niche occupied by *Nerudia* representatives is similar to other ninetines. This was done through a second PCA (see parameters in Tables S4, S5), using the extracted values for 338 points of occurrences, including 47 of *Nerudia* and 291 of other Ninetinae species (Supporting Information, Table S6). This list was assembled based on the literature and on unpublished records (B.A. Huber & L. S Carvalho, unpubl. data). A third PCA was carried out using the extracted values for the 21 predictor layers for the localities of the terminals used in the neighbour joining and phylogenetic analyses (Supporting Information, Tables S7, S8, Fig. S8). The principal components of this PCA were used to test if there is a phylogenetic signal related to the environmental niche occupied by each taxon (Supporting Information, Table S9). This analysis is preliminary in the sense that our *COI* trees may not adequately reflect phylogenetic relationships. The analysis was carried out by calculating the λ parameter (Pagel, 1999), with the function 'phylosig' of the R package 'phytools' (Revell, 2012). Pagel's λ varies from 0 (when a trait evolves independently to the phylogeny) to 1 (when a trait evolves according to Brownian motion) (Diniz-Filho *et al.*, 2012).

RESULTS

TAXONOMY

NERUDIA HUBER, 2000

Nerudia Huber, 2000: 87. Type species: *Nerudia atacama* Huber, 2000.

Diagnosis: Small (total body length 1.4–1.9) eight-eyed pholcids with relatively long legs (compared to most other Ninetinae; leg 1 usually > 3 × total body length; only shorter in the new species *N. centaura*), with simple procursus (without dorsal flap, not strongly

elongated), paired male cheliceral modifications (strong hairs or pair of apophyses), with stridulatory files on male chelicerae, with simple main (anterior) epigynal plate and large posterior epigynal plate, without or with simple (i.e. not tube-like) median receptacle-like structure in female internal genitalia.

Description

Male. Measurements: Total body length 1.4–1.9, carapace width 0.6–0.8. Legs relatively long (compared to other Ninetinae), tibia 1 usually ~1.1–2.0 (longer in the new species *N. rocio*, single male: 2.5); tibia 1 L/d 14–31; metatarsus 1 length ~1.0–1.2 × tibia 1 length; tibia 2 always shorter than tibia 4 (tibia 2/tibia 4: 0.8–0.9).

Colour: Live specimens pale ochre-grey to light brown (Fig. 1); carapace often with darker median mark; abdomen colour variable, often darker than prosoma, only in *N. centaura* distinctively lighter than prosoma (Fig. 1C), without or with indistinct marks; legs without dark or light bands. Colour in ethanol similar but paler.

Body: Ocular area barely raised, eight eyes, AME relatively large (diameter: 35–50 µm, i.e. 60–85% of PME diameter). Carapace without or with indistinct thoracic groove (cf. Figs 7B, 11A, 29A). Clypeus usually barely modified, rim slightly more sclerotized than in female, in *N. nono* also more bulging than in female, in some species with pair of shallow indentations for genital bulbs at rest (Figs 7A, 11B). Sternum wider than long, usually with pair of variably distinct anterior processes near leg coxae 1, processes apparently without pores (Fig. 29G); sternum processes absent in *N. centaura*. Abdomen globular; four epiandrous spigots arranged in two pairs (Figs 11F, 29H); ALS with seven spigots each (Fig. 30G): one strongly widened spigot, one long pointed spigot and five cylindrical spigots (one of which is unusually large); PMS with two short, pointed spigots (Fig. 30G); PLS without spigots (cf. Fig. 12D).

Chelicerae: Usually with pair of simple frontal apophyses (e.g. Fig. 5G, H; absent in *N. trigo*), tip often flat, i.e. wide in frontal view, pointed in lateral view (Fig. 11C, D); often with patches or brushes of stronger hairs; with stridulatory files on variably distinct lateral protrusions (Figs 11B, C, 29C).

Palps: Coxa unmodified; trochanter with indistinct ventral projection; femur cylindrical, slightly widened distally, proximally without or with low retrolateral hump, with prolateral stridulatory pick (modified hair; Fig. 29E); patella short; tibia globular, with two trichobothria; palpal tarsal organ raised, capsulate (Figs 7E, 11E, 30C), with small opening (diameter of opening ~1.6–1.9 µm); procursus simple, without dorsal flap, not strongly elongated, straight or bent towards dorsal, tip with complex cuticular microstructure (Fig. 29B); genital bulb with simple ventral apophysis and dorsal embolus (e.g. Fig. 5F).

Legs: Without spines and curved hairs; usually with short vertical hairs in higher density on tibia 1 or tibiae 1 and 2 (Figs 8A, B, 30A; length of hairs ~20 µm). Trichobothria in usual arrangement: three on each tibia (except tibia 1: prolateral trichobothrium absent), one on each metatarsus, slightly feathered (Fig. 30B); length of dorsal trichobothrium on tibia 1: ~80 µm; retrolateral trichobothrium of tibia 1 in distal position (at 58–70% of tibia length). Metatarsi and tarsi with dorsal rows of tiny pores with cuticular rim (diameter of opening ~0.6 µm; cf. Fig. 18C, D). Tarsus 1 with six to eight pseudosegments, distally distinct, proximally usually indistinct; tarsus 4 distally with one comb-hair on prolateral side (Fig. 12F); leg tarsal organs small, capsulate (Fig. 30E), with small opening (diameter of opening ~ 1.2–1.6 µm); three claws (Figs 12E, 18G).

Female: In general (size, colour, body shape), similar to male but chelicerae without stridulatory files (Figs 7D, 24F, 29D), sternum without pair of anterior humps, palpal tarsal organ only weakly raised (Figs 12A, B, 24B), and leg tibiae with usual low number of short vertical hairs; legs either slightly shorter than in males or of same length (only in the new species *N. ola* with reasonable sample size: male/female tibia 1 length: 1.08). Spinnerets, leg pores, leg tarsal organs and comb-hairs as in male (Figs 7F, 8D–F, 18B, H, 24H, 30E, H). Main (anterior) epigynal plate rectangular to trapezoidal or semi-circular, weakly protruding, posteriorly straight or with variably distinct median indentation (e.g. Figs 17A, 32A); posterior plate simple, short but wide, usually wider than anterior plate. Internal genitalia simple, sometimes with distinct median receptacle-like structure (e.g. Figs 6B, 10B), apparently without or with indistinct pair of pore plates (arrows in Figs 10B, 14B, 23B).

Relationships: The molecular analysis of Eberle *et al.* (2018) included only a single species of *Nerudia* ('*N. Mich20*' = *N. ola*), which was placed (with moderate support) as sister of the geographically close *Gertschiola macrostyla* (Mello-Leitão, 1941). Both genera together were placed in a clade with further South American and Old World Ninetinae. The present paper is not primarily focused on phylogeny and our sampling does not include the type species *N. atacama*, but our *COI* and karyotype data support the sister-group relationship between *Nerudia* and *Gertschiola*

(see Fig. 2; Supporting Information, Figs S1, S2; and section on karyology). Preliminary analyses of Ninetinae relationships based on hybrid enrichment data (G. Meng *et al.*, unpublished data) also support this sister-group relationship. Our new SEM data also confirm the position of *Nerudia* among Ninetinae (in particular the small opening of the tarsal organs).

In addition, the *COI* data suggest the existence of three species groups within *Nerudia* that we consider useful as working hypotheses: (1) the *ola* group, including the new species *N. guirnalda*, *N. nono*, *N. ola* and *N. trigo*; (2) the *centaura* group, including the new species *N. centaura*, *N. rocio* and the undescribed *N.* 'Arg23a'; and (3) the *poma* group, including the new species *N. hoguera*, *N. poma* and the undescribed *N.* 'Arg163'. One new species, *N. colina*, appears isolated. The three groups appear neither supported nor contradicted by morphology. The type species *N. atacama* and the new *N. flecha* cannot be assigned with confidence to any of these groups (no fresh material suitable for sequencing is available of these two species).

Distribution: Nerudia is widespread in the Argentinean and Chilean Andes, ranging at least from 24°S to 31°S, and in the east up to the Córdoba mountain ranges in central Argentina (Fig. 3). See biogeographic analysis for further details and distribution modelling.

Natural history: Most species were found in relatively arid environments (Fig. 45A–F), and some seem to

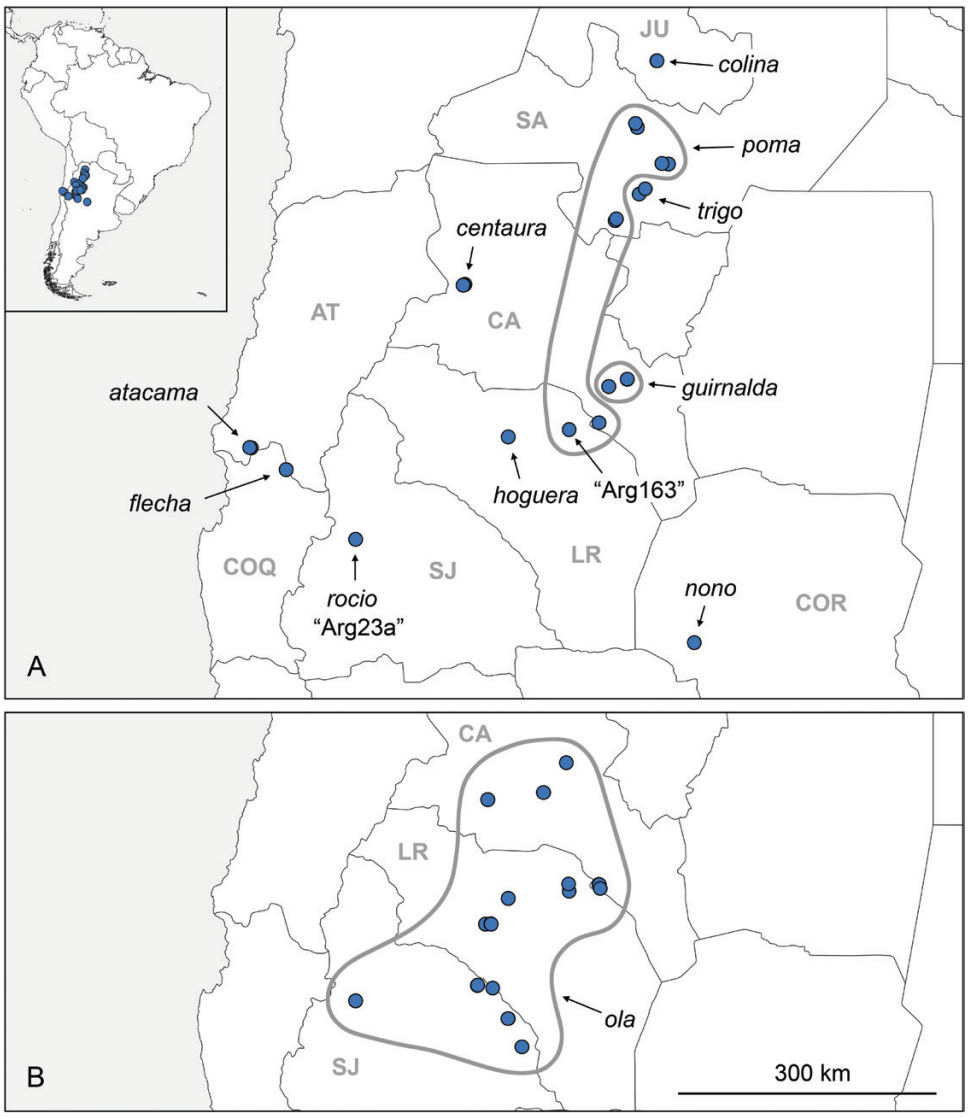

Figure 3. Known distribution of *Nerudia* in South America (inset) and of individual species. Regions in Chile: AT, Atacama; COQ, Coquimbo. Provinces in Argentina: CA, Catamarca; COR, Córdoba; JU, Jujuy; LR, La Rioja; SA, Salta; SJ, San Juan.

tolerate extremely dry and cold conditions (see Natural history section of *N. centaura* and biogeographical analysis for further details). In a few cases, *Nerudia* has been found in relatively humid forested areas, e.g. *N. guirnalda* at El Rodeo or *N. ola* and *N. poma* at Chumbicha (both: Argentina, Catamarca). Most specimens were found by turning stones and rocks. Two species (*N. poma* and *N. trigo*) were also found on the ceilings of small cave-like shelters composed of large rocks and boulders. *Nerudia nono* was collected by turning stones of a loosely built wall situated in the sun (Fig. 45F). When disturbed, the spiders either remained more or less motionless or they ran rapidly a few centimetres over the rock surface; they seemed reluctant to drop to the ground. Webs were not seen in the field, but at least *N. nono* was observed to build flimsy webs in small containers in the laboratory. In most visited localities, only one species of *Nerudia* was found; in a few cases, two or even three species were found at a single locality, apparently in identical microhabitats. Other pholcid spiders sharing the microhabitats of *Nerudia* were *Gertschiola macrostyla* and *Guaranita* Huber, 2000 spp. (Ninetinae), *Chibchea* Huber, 2000 spp. and other small undescribed representatives of Modisiminae, and an undescribed species of *Metagonia* Simon, 1893 (Pholcinae). Egg sacs were slightly flattened (but never reduced to a single layer of eggs as in *Guaranita*) and consisted of about eight to 18 eggs.

Composition: The genus now includes 11 described species, ten of which are newly described below.

NERUDIA COLINA HUBER SP. NOV.

(FIGS 1A, 4–8)

Zoobank registration: urn:lsid:zoobank.org:act:7B358FEA-C1CA-4841-BBDA-DF46379B51C0.

Diagnosis: Distinguished from known congeners by shapes of procursus (Fig. 5A–C; with subdistal ventral sclerite, partly semi-transparent flat tip) and by armature of male chelicerae (Fig. 5G, H; frontal apophysis with wide and flat tip, in frontal view slightly bifid; similar to *N. poma*); from some congeners also by bulbal processes (Fig. 5D–F; ventral apophysis

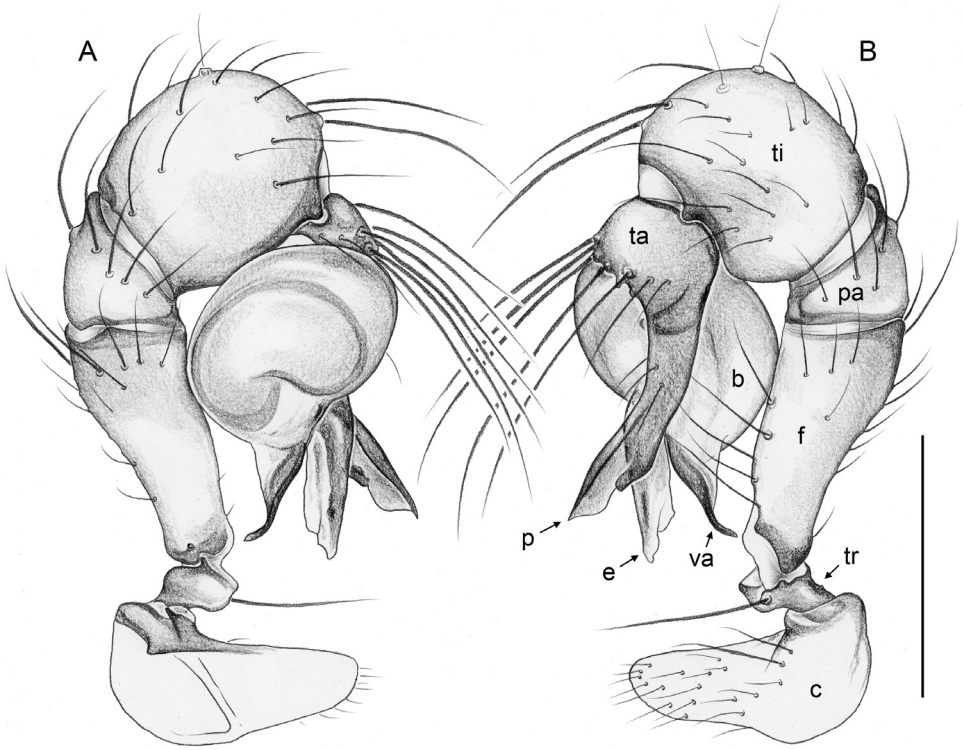

Figure 4. *Nerudia colina* sp. nov.; paratype male from Argentina, Jujuy, between San Salvador and Purmamarca (ZFMK Ar 23883). Left palp, prolateral (A) and retrolateral (B) views. Abbreviations: b, genital bulb; c, coxa; e, embolus; f, femur; p, procursus; pa, patella; ta, tarsus; ti, tibia; tr, trochanter; va, ventral apophysis. Scale line: 0.3 mm.

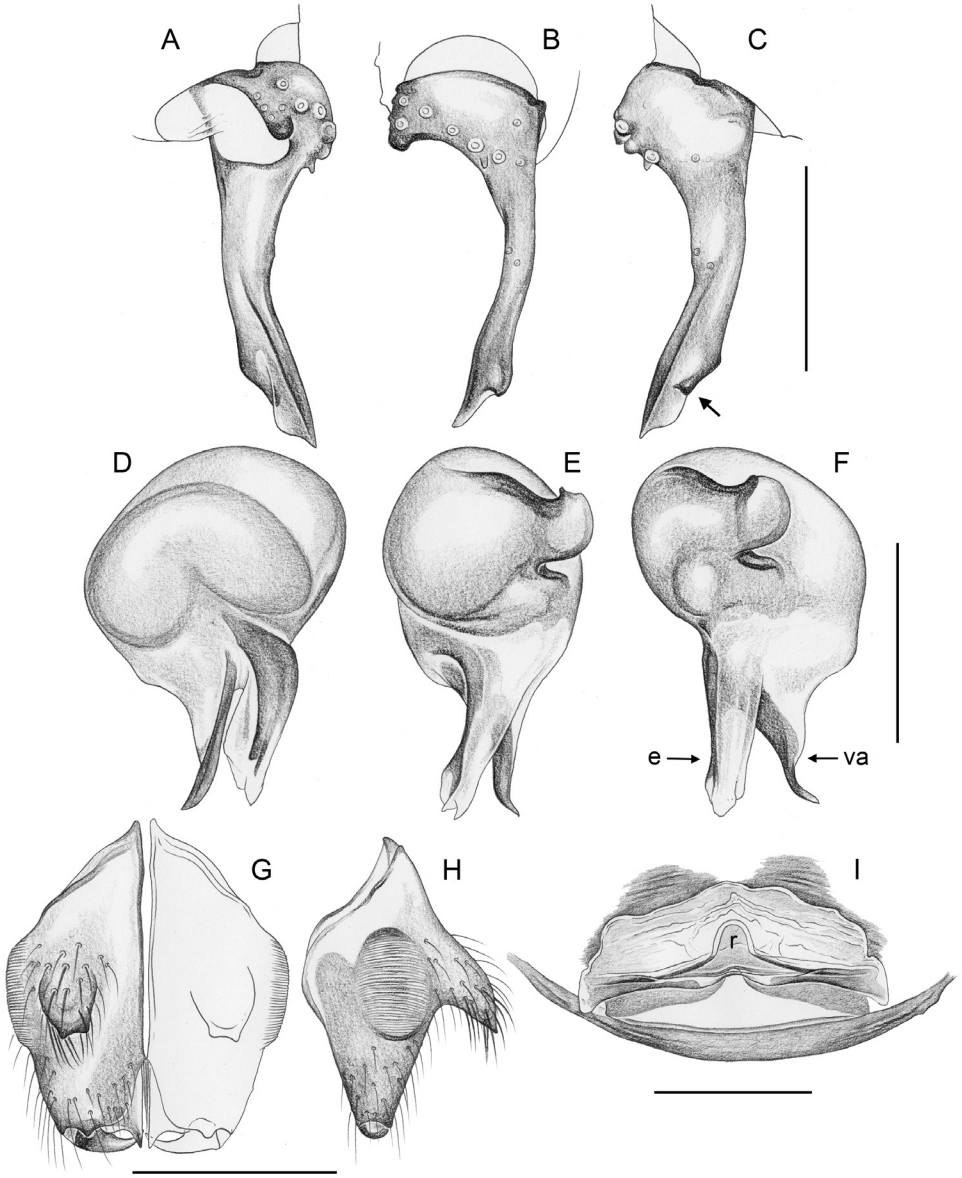

Figure 5. *Nerudia colina* sp. nov.; paratype male and paratype female from Argentina, Jujuy, between San Salvador and Purmamarca (ZFMK Ar 23883). A–C, left male palpal tarsus and procursus, prolateral, dorsal, and retrolateral views (arrow points at distinctive subdistal sclerite). D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Abbreviations: e, embolus; r, 'receptacle'; va, ventral apophysis. Scale lines: 0.2 mm.

distally slender, curved towards ventral, same length as embolus) and by epigynum and female internal genitalia (Figs 5I, 6; epigynal plate with indistinct posterior and anterior median indentations; internal genitalia with posteriorly wide open 'receptacle'; similar to *N. guirnalda*).

Type material: ARGENTINA – **Jujuy**: • ♂ holotype; between San Salvador and Purmamarca, 'site 2'; 23.8849° S, 65.4613° W; 2150 m a.s.l.; 16–17 March 2019; B. A. Huber and M. A. Izquierdo leg.; LABRE-Ar 584 • 3 ♂♂, 4 ♀♀, paratypes (one male used for SEM); same data as holotype; ZFMK Ar 23883.

Other material examined: ARGENTINA – **Jujuy**: • 14 ♀♀, in pure ethanol (two females used for SEM; three female prosomata used for molecular study); same data as holotype; ZFMK Arg178 • 2 ♀♀; same data as

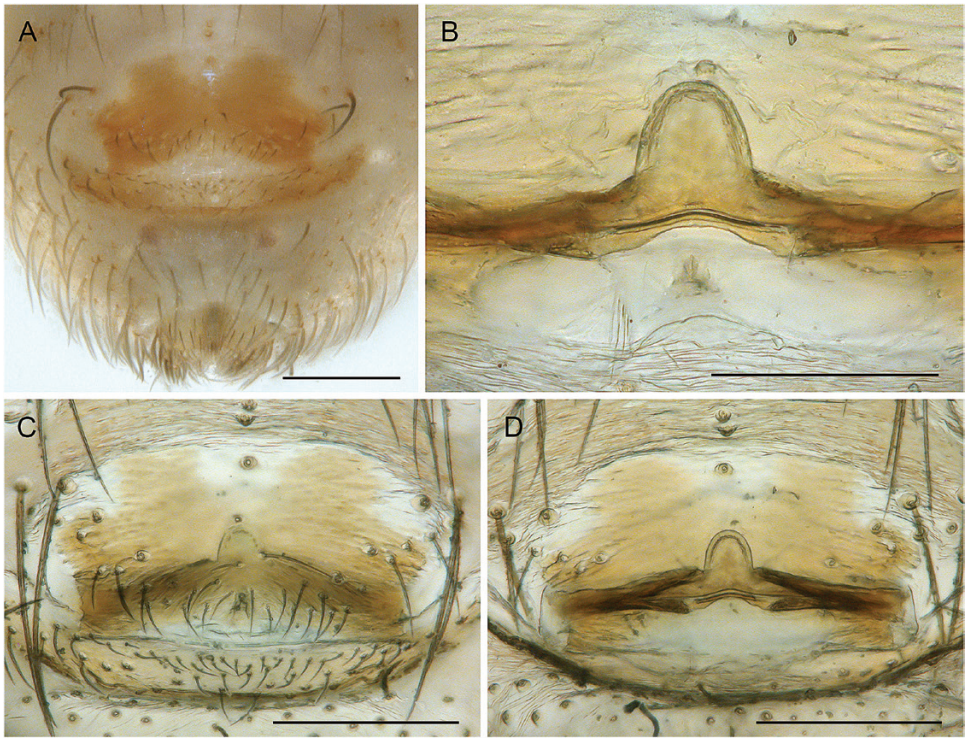

Figure 6. *Nerudia colina* sp. nov.; paratype female from Argentina, Jujuy, between San Salvador and Purmamarca (ZFMK Ar 23883). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia. C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

holotype; LABRE-Ar 537 • 1 ♂, 3 ♀♀, in pure ethanol; between San Salvador and Purmamarca, 'site1'; 23.8866° S, 65.4588° W; 2100 m a.s.l.; under rocks; 16–17 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; LABRE-Ar 549 • 2 ♀♀, 1 juv., in pure ethanol; same data as preceding; LABRE-Ar 559.

Etymology: The species epithet *colina* (Spanish for 'hill') is taken from Pablo Neruda's poem 'Poema 1'; noun in apposition.

Description

Male (holotype). Measurements: Total body length 1.75, carapace width 0.72. Distance PME–PME 70 µm; diameter PME 50 µm; distance PME–ALE 20 µm; distance AME–AME 15 µm; diameter AME 40 µm. Leg 1: 5.94 (1.60 + 0.27 + 1.60 + 1.77 + 0.70), tibia 2: 1.37, tibia 3: 1.10, tibia 4: 1.50; tibia 1 L/d: 21.

Colour (in ethanol): Prosoma and legs mostly pale ochre-grey; ocular area, thoracic groove, and clypeus light brown; legs without dark rings; abdomen monochromous grey.

Body: Habitus as in *N. poma* (cf. Fig. 1B). Ocular area barely raised. Carapace with indistinct thoracic groove. Clypeus unmodified except for pair of indentations for genital bulb at rest (Fig. 7A). Sternum wider than long (0.52/0.36), with pair of distinct anterior processes near coxae 1. Abdomen globular.

Chelicerae: As in Figure 5G, H; pair of frontal apophyses directed downwards, with slightly bifid tip slightly flattened in lateral view (Fig. 7A, C); stridulatory files on pair of low lateral protrusions (Fig. 7C).

Palps: As in Figure 4; coxa unmodified; trochanter with indistinct ventral projection; femur cylindrical, only slightly widened distally, proximally with indistinct retrolateral hump and prolateral stridulatory pick (modified hair); patella short; tibia globular; procursus (Fig. 5A–C) simple, in lateral view slightly directed towards dorsal, with distinctive subdistal ventral sclerite and partly semi-transparent flat tip; genital bulb (Fig. 5D–F) with ventral apophysis distally slender, curved towards ventral, embolus partly membranous.

Legs: Without spines and curved hairs; with slightly higher than usual density of short vertical hairs on tibia 1 (Fig. 8A, B); retrolateral trichobothrium of tibia 1 at 70%; prolateral trichobothrium absent on tibia 1; tarsus 1 with seven to eight pseudosegments, distally distinct.

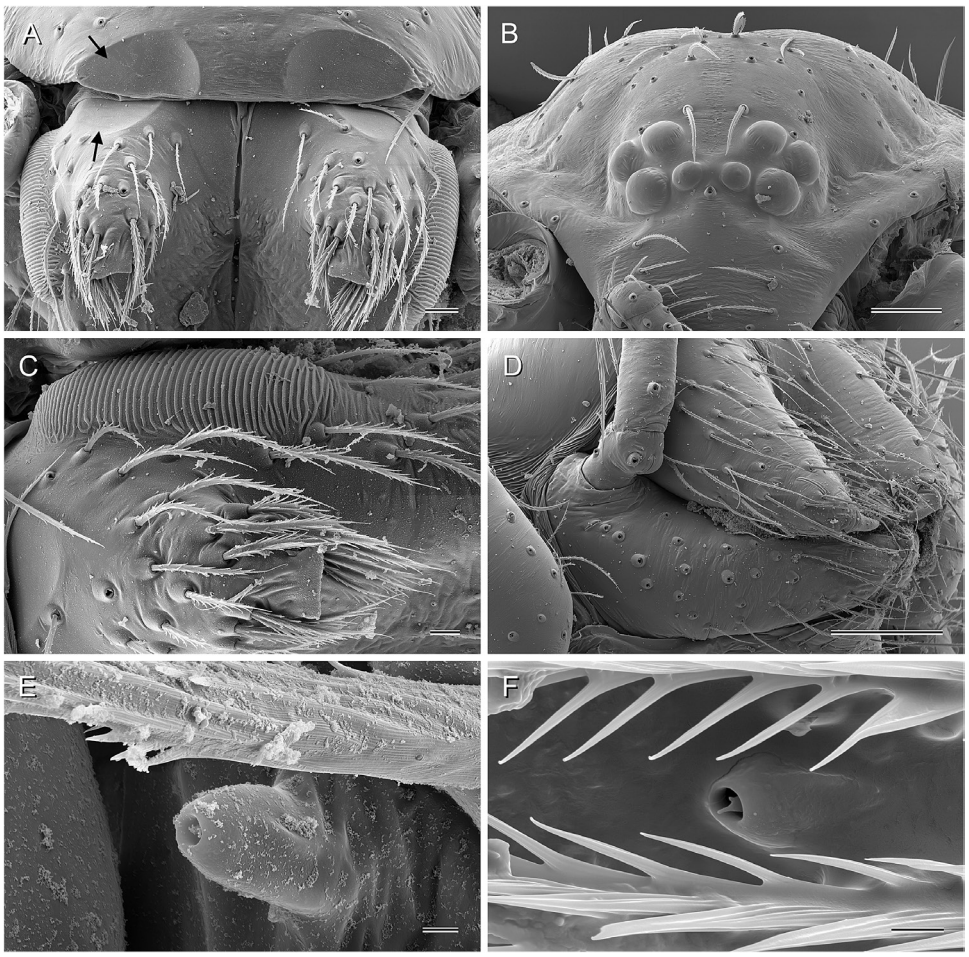

Figure 7. *Nerudia colina* sp. nov.; male and female from Argentina, Jujuy, between San Salvador and Purmamarca (ZFMK Ar 23883 and ZFMK Arg178). A, male clypeus and chelicerae, frontal view (arrows point at indentations for genital bulb at rest). B, female prosoma, frontal view. C, left male chelicera, frontal view. D, female chelicerae and right palp, proximal segments, lateral view. E, male palpal tarsal organ. F, female tarsal organ on tarsus 1. Scale lines: 20 µm (A), 100 µm (B, D), 10 µm (C), 2 µm (E, F).

Variation (male): Tibia 1 in five males (including holotype): 1.50–1.73 (mean 1.62). Abdomen variably dark.

Female: In general, similar to male but sternum without pair of anterior humps and tibiae with few vertical hairs. Tibia 1 in nine females: 1.37–1.63 (mean 1.49). Epigynum (Fig. 6A) anterior plate weakly protruding, with anterior and indistinct posterior indentations; posterior plate large, simple. Internal genitalia (Figs 5I, 6B–D) with posteriorly wide open 'receptacle'.

Distribution: Known only from type locality in Jujuy, Argentina (Fig. 3).

Natural history: The spiders were found by turning stones and rocks on an arid slope (Fig. 45A). They shared the microhabitat with a second species of Ninetinae, an unidentified species of *Guaranita*. They seemed to prefer large stones and rocks that were close to vegetation. When disturbed, they ran rapidly but remained on the rock. No webs were seen.

NERUDIA POMA HUBER SP. NOV.

(FIGS 1B, 9–12)

Zoobank registration: urn:lsid:zoobank.org:act:425882CF-A239-4258-BADC-CD03F44F3C54.

Nerudia atacama (misidentification; see Note below) – Torres *et al.*, 2015: 5, fig. 4C, D.

Diagnosis: Distinguished from known congeners by shapes of procursus (Fig. 9A–C; distally slender, slightly bent towards dorsal and divided into sclerotized dorsal and transparent ventral part; similar to *N. hoguera*)

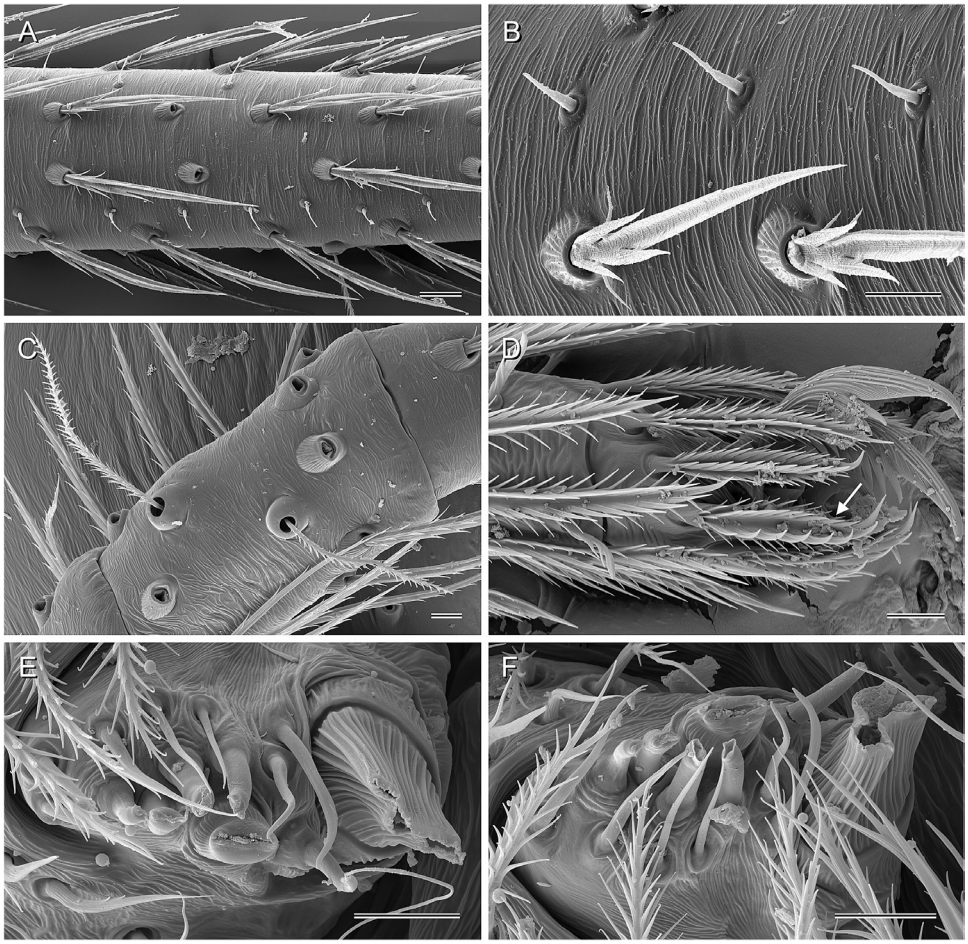

Figure 8. *Nerudia colina* sp. nov.; male and female from Argentina, Jujuy, between San Salvador and Purmamarca (ZFMK Ar 23883 and ZFMK Arg178). A, detail of male left tibia 1, prolateral view. B, hairs on male tibia 1. C, female palpal tibia, dorsal view. D, female left tarsus 4, prolateral view (arrow points at comb-hair). E, F, female ALS. Scale lines: 20 µm (A), 10 µm (B–F).

and by armature of male chelicerae (Fig. 9G, H; frontal apophyses with wide, flattened tip; set with strong hairs); also by shapes of bulbal processes (Fig. 9D–F; ventral apophysis short, slightly curved towards ventral, same length as embolar process) and by epigynum and female internal genitalia (Figs 9I, 10; epigynal plate rectangular, posterior margin weakly curved; internal genitalia with cylindrical 'receptacle', with median sclerite similar to *N. hoguera*).

Type material: ARGENTINA – **Salta**: • ♂ holotype; ~15 km NW Campo Quijano; 24.7918° S, 65.7297° W; 2020 m a.s.l.; 19 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; *LABRE-Ar 585* • 7 ♂♂, 5 ♀♀, paratypes (one male used for SEM; two male abdomens used for karyotype study; two males and three females used for µ-CT study); same data as holotype; ZFMK Ar 23884.

Other material examined: ARGENTINA – **Salta**: • 9 ♀♀, in pure ethanol (one female used for SEM; three prosomata used for molecular study); same data as holotype; ZFMK Arg184 • 2 ♂♂, 2 ♀♀; Cabra Corral, 'site 3', ~3.5 km SE of dam; 25.2907° S, 65.3057° W; 1000 m a.s.l.; 21 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23885 • 3 ♀♀, 1 juv., in pure ethanol; same data as preceding; ZFMK Arg195 • 2 ♂♂, 2 ♀♀ (one male and one female used for µ-CT study); Cabra Corral, 'site 4', W end of bridge; 25.2837° S, 65.3939° W; 1050 m a.s.l.; under rocks; 21–22 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23886 • 1 ♂, 3 ♀♀, 3 juvs, in pure ethanol; same data as preceding; ZFMK Arg197 • 1 ♂, 3 ♀♀, 9 juvs; same data as preceding; LABRE-Ar 540, 565. **Catamarca**: • 1 ♂; ~5 km NW Chumbicha, near Balneario El Caolín, 'site 2'; 28.8109° S, 66.2500° W; 640 m a.s.l.; steep rock field in forest; 28–29 Mar. 2019; B. A. Huber and M. A.

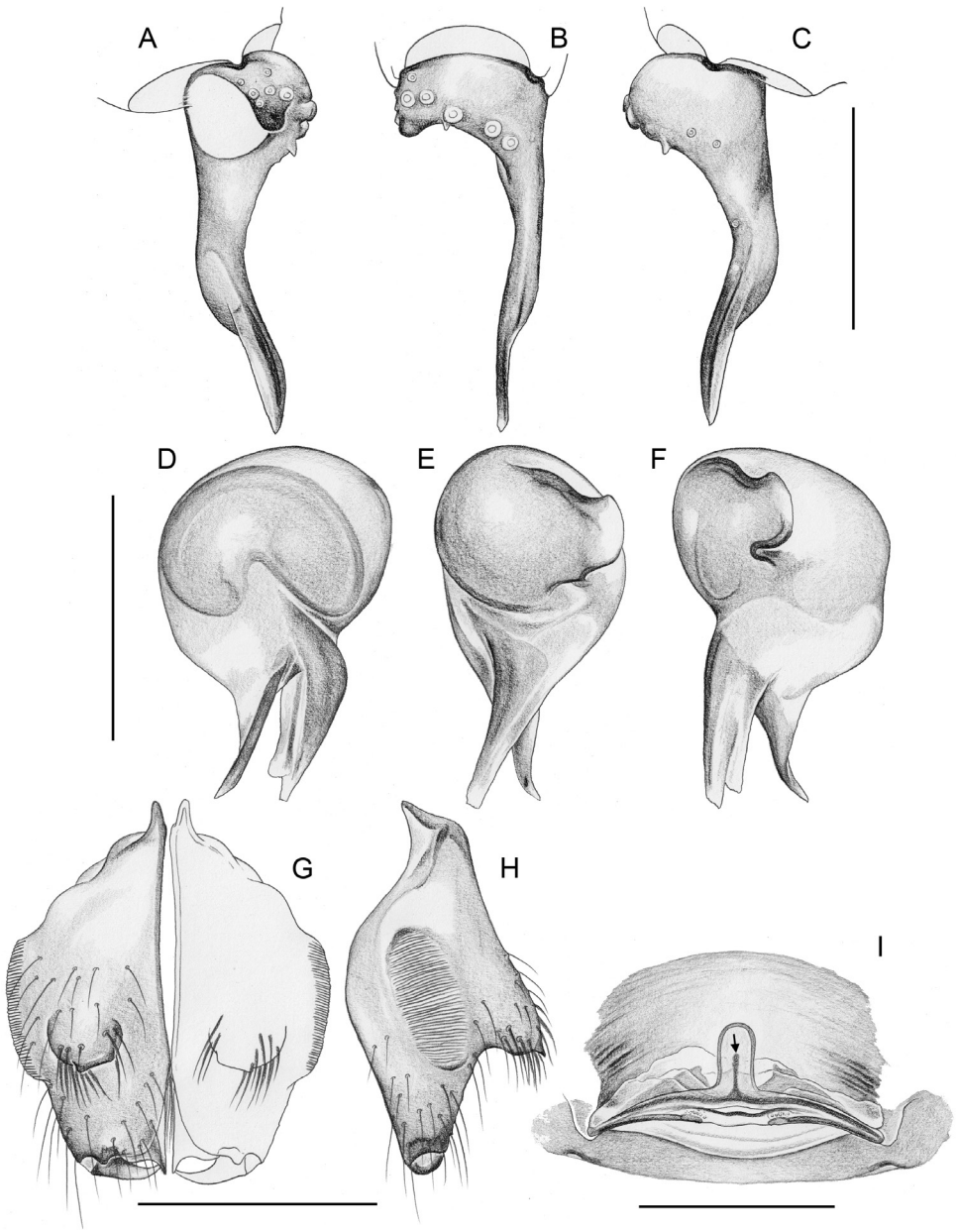

Figure 9. *Nerudia poma* sp. nov.; paratype male and paratype female from Argentina, Salta, 15 km NW Campo Quijano (ZFMK Ar 23884). A–C, left male palpal tarsus and procursus, prolateral, dorsal, and retrolateral views. D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view (arrow points at median sclerite). Scale lines: 0.2 mm.

Izquierdo leg.; ZFMK Ar 23887. **La Rioja**: • 1 ♂, in pure ethanol; SE Aimogasta, 'site 2'; 28.9015° S, 66.6538° W; 755 m a.s.l.; under rocks; 10 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; LABRE-Ar 558.

Assigned tentatively (only females available, identity uncertain): ARGENTINA – **Salta**: • 2 ♀♀; ~5 km W Cafayate, 'site 1'; 26.0641° S, 66.0294° W; 2060 m a.s.l.; on rocks in small shelters; 24 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23888 • 5 ♀♀, in pure ethanol; same data as preceding; ZFMK Arg208 • 4 ♀♀, 5 juvs, in pure ethanol; same data as preceding; LABRE-Ar 557 • 1 ♀, 1 juv.; Chuscha, 6 km NW Cafayate; ~26.035° S, 66.017° W; ~1900 m a.s.l.; 17 Jul. 1995; M. Ramírez and P. Goloboff leg.; MACN 20094.

Note: We have not re-examined the two male specimens from Salta Province assigned to *N. atacama*

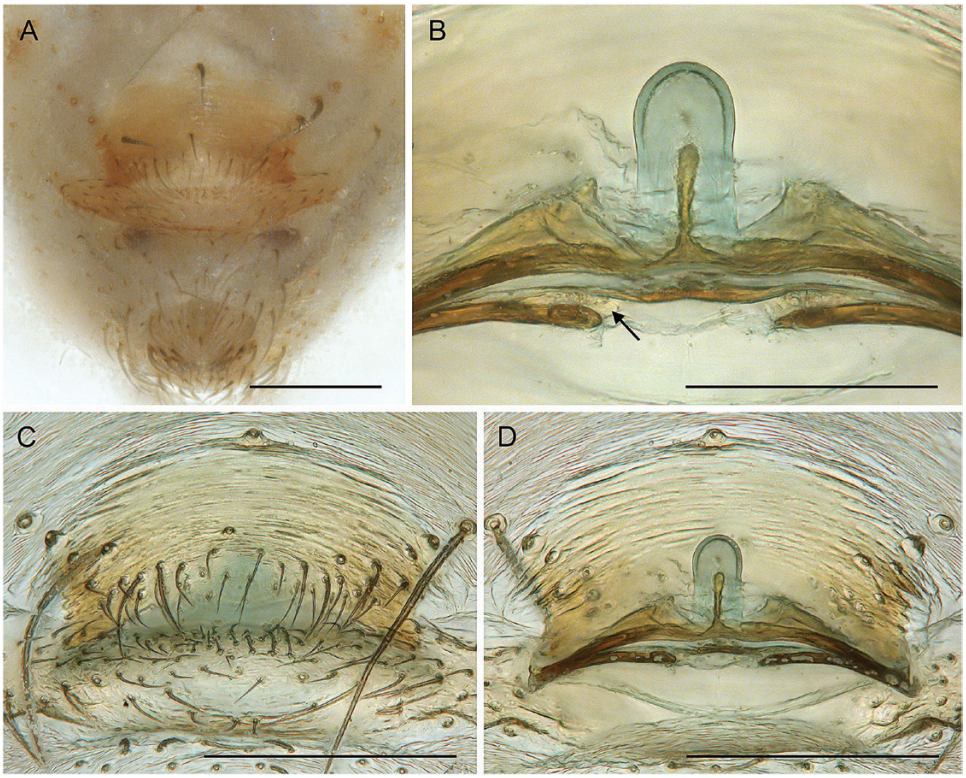

Figure 10. *Nerudia poma* sp. nov.; paratype female from Argentina, Salta, 15 km NW Campo Quijano (ZFMK Ar 23884). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia. C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

by Torres *et al.* (2015). However, the types of the new species described herein originate from 6.5 km SE of the locality reported in Torres *et al.* (2015), in the same river valley at a similar elevation. In addition, the procursus shown in Torres *et al.* (2015: fig. 4D) agrees well with the one shown in Figure 9C.

Etymology: The species epithet *poma* (Spanish for 'apple') is taken from Pablo Neruda's poem 'Vírese'; noun in apposition.

Description

Male (holotype). Measurements: Total body length 1.44, carapace width 0.64. Distance PME–PME 80 µm; diameter PME 60 µm; distance PME–ALE 20 µm; distance AME–AME 15 µm; diameter AME 35 µm. Leg 1: 5.26 (1.45 + 0.20 + 1.43 + 1.53 + 0.65), tibia 2: 1.20, tibia 3: 1.00, tibia 4: 1.40; tibia 1 L/d: 20.

Colour (in ethanol): Prosoma and legs pale ochre-yellow; with darker Y-mark on carapace; legs without dark rings; abdomen light grey.

Body: Habitus as in Figure 1B. Ocular area barely raised. Carapace with indistinct thoracic groove. Clypeus unmodified, only at rim slightly sclerotized. Sternum wider than long (0.48/0.38), with pair of low anterior processes near coxae 1. Abdomen globular.

Chelicerae: As in Figure 9G, H; with pair of short frontal apophyses slightly pointing downward and set with strong hairs, with wide, flattened tip pointed in lateral view (Fig. 11B–D); stridulatory files on pair of low lateral protrusions (Fig. 11B, C).

Palps: In general, similar to *N. colina* (cf. Fig. 4); coxa unmodified; trochanter with indistinct ventral projection; femur cylindrical, slightly widened distally, proximally with indistinct retrolateral hump and prolateral stridulatory pick (modified hair), femur length/width: 1.91; patella short; tibia globular (length/width: 1.15); procursus simple (Fig. 9A–C), distally slender, curved towards dorsal, divided into sclerotized dorsal and transparent ventral part; genital bulb with ventral apophysis short, slightly curved towards ventral; embolus partly membranous.

Legs: Without spines and curved hairs; with vertical hairs in two rows (prolateral, retrolateral) on tibia 1 only; retrolateral trichobothrium of tibia 1 at 67%; prolateral trichobothrium absent on tibia 1; tarsus

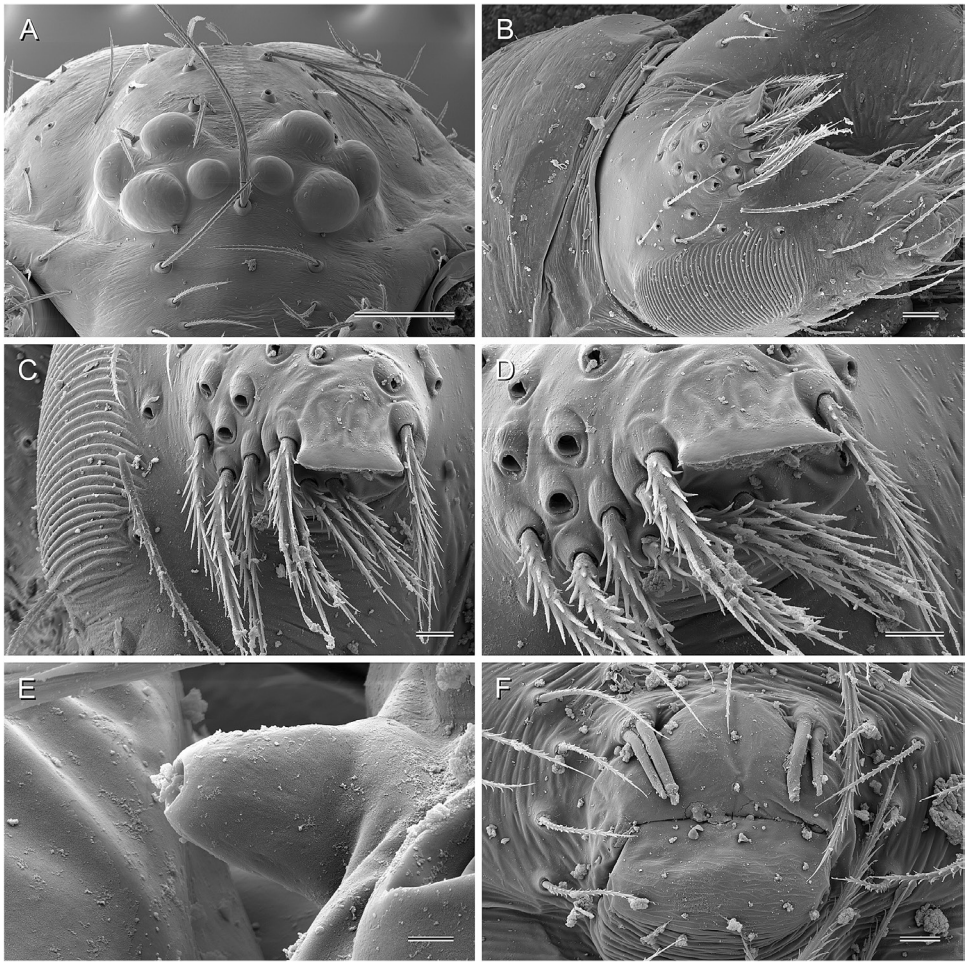

Figure 11. *Nerudia poma* sp. nov.; male and female from Argentina, Salta, 15 km NW Campo Quijano (ZFMK Ar 23884, ZFMK Arg184). A, female prosoma, frontal view. B, male clypeus and chelicerae, oblique distal view. C, D, right male chelicera, frontal views. E, male palpal tarsal organ. F, male gonopore. Scale lines: 100 µm (A), 20 µm (B), 10 µm (C, D, F), 2 µm (E).

1 with ~six to seven pseudosegments, only distally distinct.

Variation (male): Tibia 1 in nine males from Salta (including holotype): 1.27–1.50 (mean 1.41); in male from Catamarca: 1.47; in male from La Rioja: 1.35. The chelicerae and palps of the male from Catamarca appear indistinguishable from those from Salta.

Female: In general, similar to male but sternum without pair of anterior humps and tibiae with few vertical hairs. Tibia 1 in eight females: 1.30–1.50 (mean 1.42). Epigynum (Fig. 10A) anterior plate rectangular, posterior margin indented; posterior plate wide but short. Internal genitalia (Figs 9I, 10B–D) with median cylindrical 'receptacle' and median sclerite.

Distribution: Known from several localities in Salta Province, Argentina, and from one locality each in Catamarca and La Rioja (Fig. 3).

Natural history: At the type locality (Fig. 45B), the spiders were found by turning large rocks. At disturbance they started to run over the rock surface but did not drop to the ground. They shared the microhabitat with *Chibchea araona*(?). At Cabra Corral 'site 3', the spiders were found sitting on the undersides of large boulders, i.e. on the ceilings of small cave-like spaces under the rocks. At Cabra Corral 'site 4', the spiders were found under small stones on the floor of a small cave/shelter. Near Chumbicha, the single male specimen was found at the same locality as *N. ola*.

NERUDIA HOGUERA HUBER **SP. NOV.**

(FIGS 13, 14)

Zoobank registration: urn:lsid:zoobank.org:act:D72E218E-77C9-4026-AF20-DE2D591CFB4F.

Diagnosis: Distinguished from known congeners by armature of male chelicerae (Fig. 13G, H;

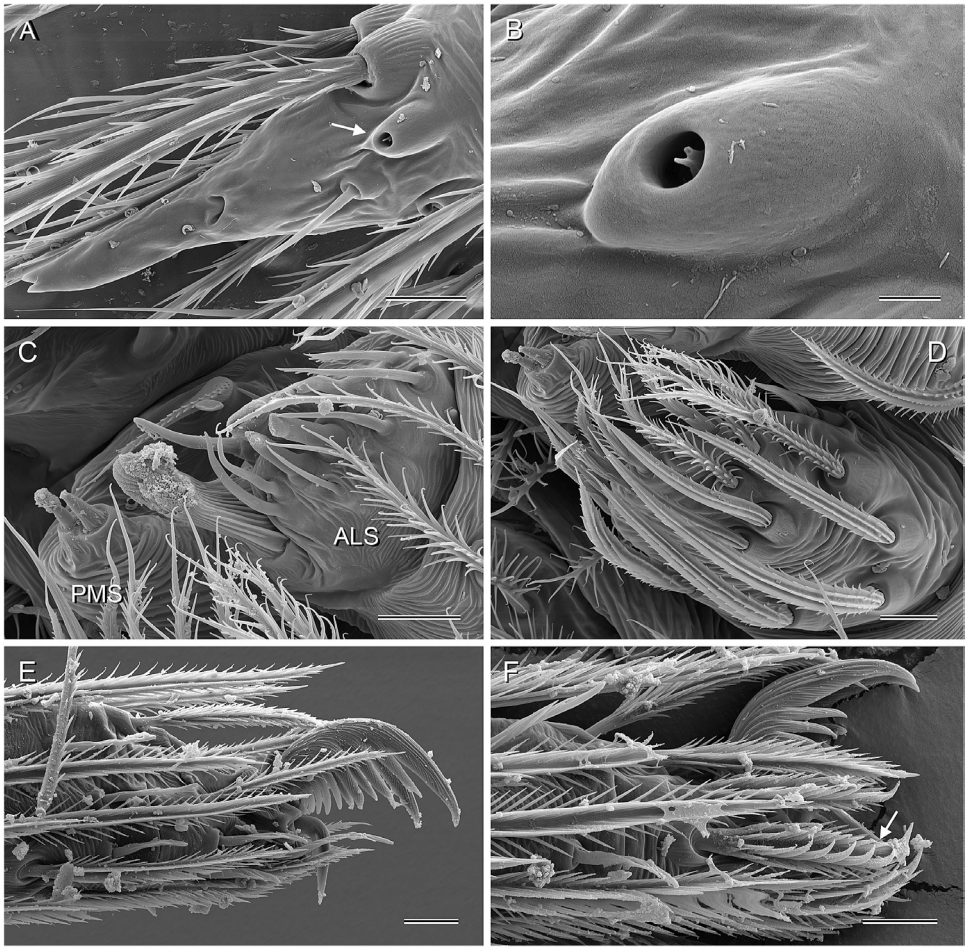

Figure 12. *Nerudia poma* sp. nov.; male and female from Argentina, Salta, 15 km NW Campo Quijano (ZFMK Ar 23884, ZFMK Arg184). A, tip of female pedipalp, dorsal view; arrow points at tarsal organ. B, female palpal tarsal organ. C, left female ALS and PMS. D, left female PLS. E, male left tarsus 1 tip, prolateral view. F, male left tarsus 4 tip, prolateral view; arrow: comb-hair. Scale lines: 10 µm (A, C–F), 2 µm (B).

frontal apophyses in lateral position, relatively long, tip flattened, i.e. wide in frontal view, pointed in lateral view), by shape of procursus (Fig. 13A–C; slender, with simple pointed tip; similar to *N. poma*), by bulbal processes (Fig. 13D–F; ventral apophysis almost straight, longer than embolus), and by epigynum and female internal genitalia (Figs 13I, 14; epigynal plate with wide posterior indentation; internal genitalia with large median 'receptacle', with median sclerite similar to *N. poma*).

Type material: ARGENTINA – **La Rioja**: • ♂ holotype; between Chilecito and Famatina; 29.0027° S, 67.4855° W; 1300 m a.s.l.; 9 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; *LABRE-Ar 586* • 2 ♀♀ paratypes; same data as holotype; ZFMK Ar 23889.

Other material examined: ARGENTINA – **La Rioja**: • 3 ♀♀ in pure ethanol; same data as holotype; ZFMK Arg159.

Etymology: The species epithet *hoguera* (from Spanish meaning 'bonfire') is taken from Pablo Neruda's poem 'Soneto 22'; noun in apposition.

Description

Male (holotype). Measurements: Total body length 1.53, carapace width 0.68. Distance PME–PME 60 µm; diameter PME 60 µm; distance PME–ALE 20 µm; distance AME–AME 15 µm; diameter AME 40 µm. Leg 1: 5.73 (1.55 + 0.25 + 1.60 + 1.73 + 0.60), tibia 2: 1.35, tibia 3: 1.10, tibia 4: 1.50; tibia 1 L/d: 23.

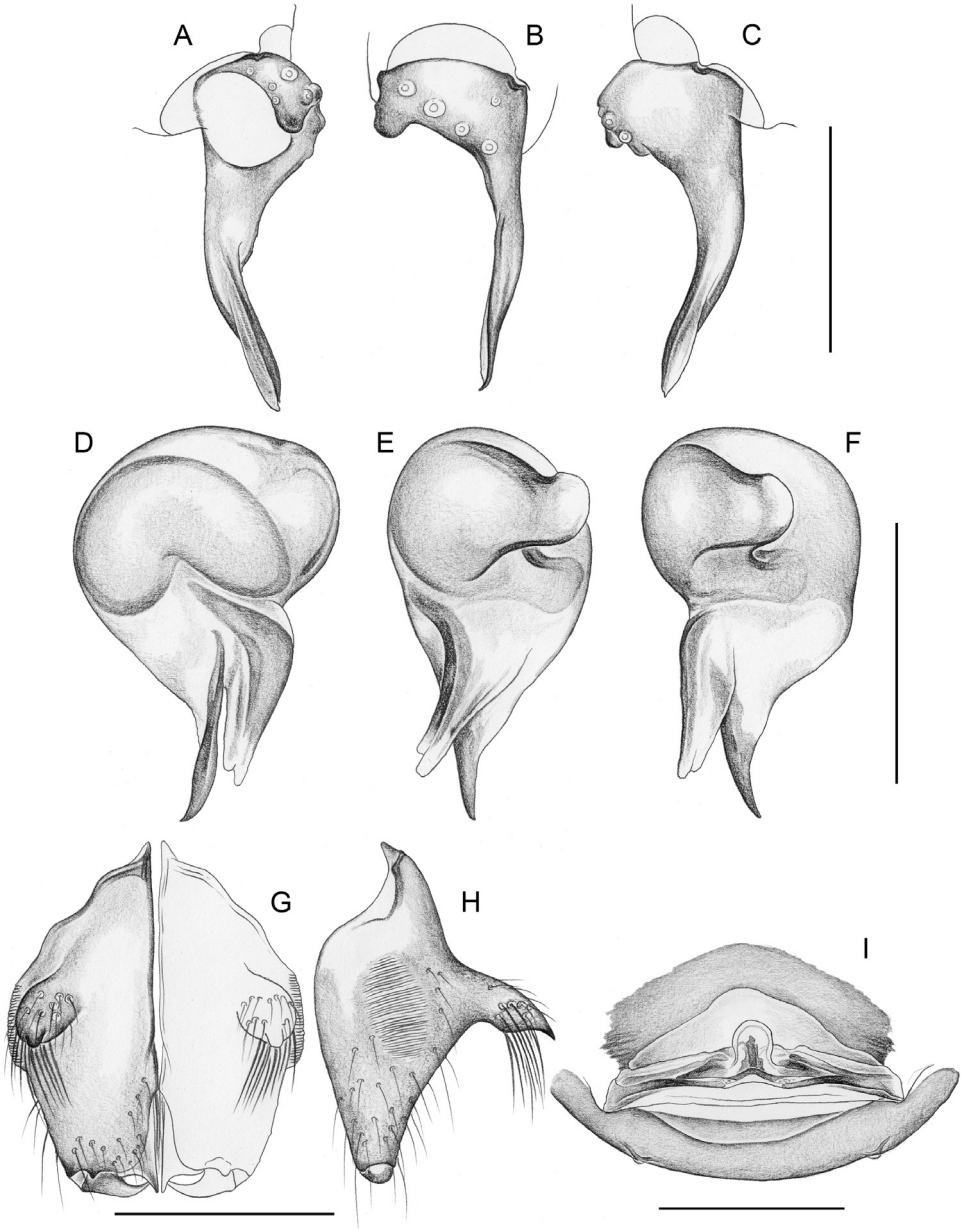

Figure 13. *Nerudia hoguera* sp. nov.; holotype male (*LABRE-Ar 586*) and paratype female (ZFMK Ar 23889) from Argentina, La Rioja, between Chilecito and Famatina. A–C, left male palpal tarsus and procursus, prolateral, dorsal, and retrolateral views. D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Scale lines: 0.2 mm.

Colour (in ethanol): Prosoma and legs pale ochre-yellow; with indistinct ochre mark medially on carapace; legs without dark rings; abdomen monochromous light grey.

Body: Habitus as in *N. poma* (cf. Fig. 1B). Ocular area barely raised. Carapace with indistinct thoracic groove. Clypeus unmodified, only at rim slightly sclerotized. Sternum wider than long (0.48/0.44), with pair of distinct anterior processes near coxae 1. Abdomen globular.

Chelicerae: As in Figure 13G, H; with pair of frontal apophyses in lateral position, directed forward, tip flattened, i.e. wide in frontal view, pointed in lateral view; stridulatory files on pair of low lateral protrusions.

Palps: In general, similar to *N. colina* (cf. Fig. 4); coxa unmodified; trochanter with indistinct ventral projection; femur cylindrical, slightly widened distally, proximally with indistinct retrolateral hump and prolateral

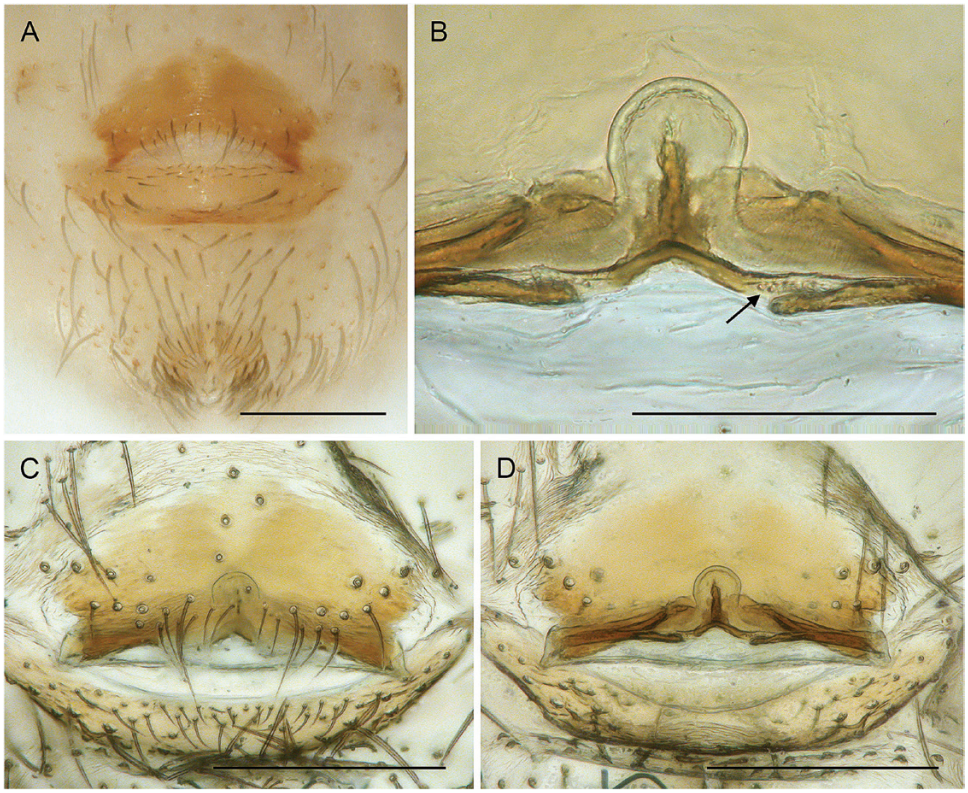

Figure 14. *Nerudia hoguera* sp. nov.; paratype female from Argentina, La Rioja, between Chilecito and Famatina (ZFMK Ar 23889). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia (arrow points at possible pore plate). C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

stridulatory pick (modified hair), femur relatively short (length/width: 1.81); patella short; tibia globular (length/width: 1.05); procursus simple (Fig. 13A–C), slender, with simple pointed tip; genital bulb with weakly curved ventral apophysis, embolus partly membranous, shorter than ventral apophysis (Fig. 13D–F).

Legs: Without spines and curved hairs; with vertical hairs in two rows (prolateral, retrolateral) proximally on tibia 1 only; retrolateral trichobothrium of tibia 1 at 65%; prolateral trichobothrium absent on tibia 1; tarsus 1 with ~eight pseudosegments, distally distinct.

Female: In general, similar to male but sternum without pair of anterior humps. Tibia 1 in five females: 1.20–1.55 (mean 1.46). Epigynum (Fig. 14A) anterior plate weakly protruding, trapezoidal, with wide posterior indentation; posterior plate large, simple. Internal genitalia (Figs 13I, 14B, D) with large median 'receptacle' and sclerite at median line.

Distribution: Known from type locality only, in Argentina, La Rioja (Fig. 3).

Natural history: The spiders were found by turning rocks in ravines on an arid slope. When disturbed, they moved slowly. They shared the microhabitat with two other Ninetinae: *Nerudia ola* and *Gertschiola macrostyla*.

NERUDIA CENTAURA HUBER **SP. NOV.**

(FIGS 1C, D, 15–18)

Zoobank registration: urn:lsid:zoobank.org:act:124FC4AB-B97D-445C-AD11-C3604843AED3.

Diagnosis: Easily distinguished from known congeners by dark coloration of prosoma (Fig. 1C, D), by shape of procursus (Fig. 16A–C; dorsal hump at basis, strongly bent towards dorsal, pointed tip), by bulbal processes (Fig. 16D–F; strong pointed ventral apophysis; embolus slightly shorter, with strong dorsal sclerite), by male chelicerae (Fig. 16G, H; strong frontal apophyses with flattened tips; stridulatory files on strong lateral protrusion), and by epigynum and female internal genitalia (Figs 16I, 17; epigynal plate with large, whitish posterior indentation angular anteriorly; internal genitalia with indistinct posterior

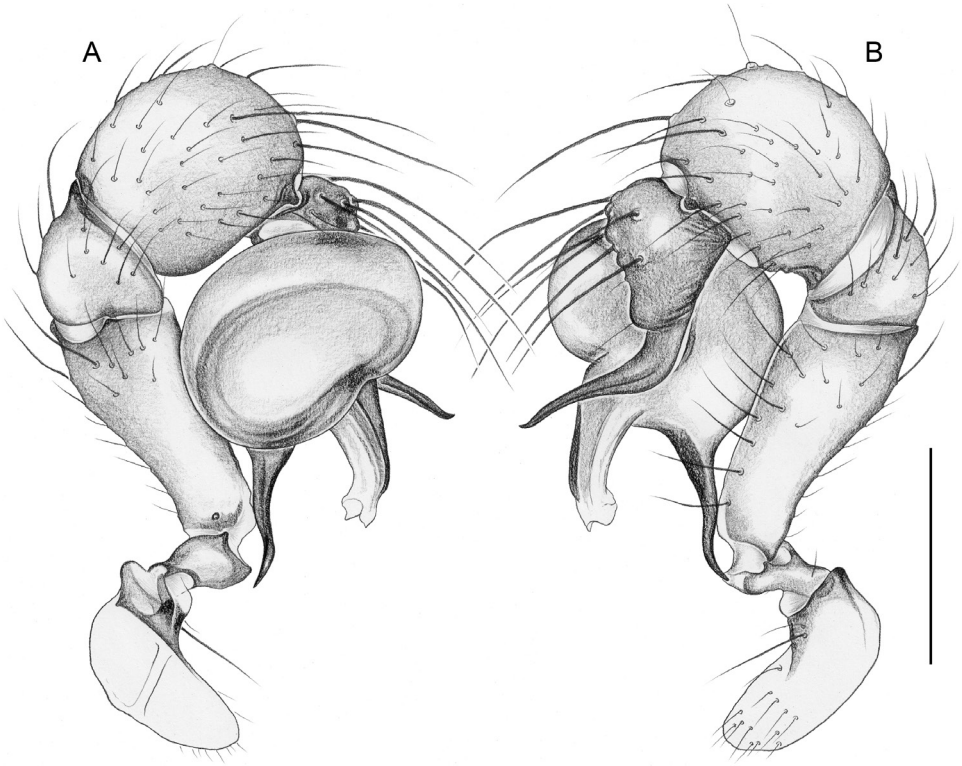

Figure 15. *Nerudia centaura* sp. nov.; non-type male from Argentina, Catamarca, 20 km E Paso de San Francisco (ZFMK Ar 23891). Left palp, prolateral (A) and retrolateral (B) views. Scale line: 0.3 mm.

'receptacle' and unique anterior tubular membranous structure).

Type material: ARGENTINA – **Catamarca**: • ♂ holotype; ~20 km E Paso de San Francisco, 'site 1'; 26.9276° S, 68.0709° W; 4180 m a.s.l.; 27 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; *LABRE-Ar 587* • 1 ♂, 2 ♀♀, paratypes; same data as holotype; ZFMK Ar 23890.

Other material examined: ARGENTINA – **Catamarca**: • 6 ♀♀, 3 juvs, in pure ethanol; same data as holotype; ZFMK Arg215 • 4 ♂♂, 6 ♀♀; same data as holotype; LABRE-Ar 526 • 3 ♀♀, 7 juvs, in pure ethanol; same data as holotype; LABRE-Ar 542 • 3 ♂♂, 5 ♀♀ (one male and two females used for µ-CT study); ~20 km E Paso de San Francisco, 'site 2'; 26.936° S, 68.090–68.095° W; 4270–4400 m a.s.l.; 27 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23891 • 6 ♀♀, 2 juvs, in pure ethanol (one female used for SEM; three female prosomata used for molecular study); same data as preceding; ZFMK Arg216 • 4 ♂♂, 1 ♀; same data as preceding; LABRE-Ar 527 • 6 ♀♀, 3 juvs, in pure ethanol; same data as preceding; LABRE-Ar 551 • 1 ♀; same data as preceding but 4450 m a.s.l.; ZFMK Ar 23892.

Etymology: The species epithet *centaura* (Spanish for 'female centaur') is taken from Pablo Neruda's poem 'Soneto 22'; noun in apposition.

Description

Male (holotype). Measurements: Total body length 1.9, carapace width 0.77. Distance PME–PME 90 µm; diameter PME 55 µm; distance PME–ALE 30 µm; distance AME–AME 20 µm; diameter AME 40 µm. Leg 1: 4.55 (1.25 + 0.25 + 1.30 + 1.30 + 0.45), tibia 2: 1.10, tibia 3: 1.00, tibia 4: 1.38; tibia 1 L/d: 14.

Colour (in ethanol): Prosoma and legs ochre to light brown; legs without dark rings; abdomen monochromous pale grey.

Body: Habitus as in Figure 1C. Ocular area barely raised. Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.56/0.48), without anterior processes. Abdomen globular.

Chelicerae: As in Figure 16G, H; pair of frontal apophyses directed downwards, with obtuse tip slightly flattened; stridulatory files on pair of distinct lateral protrusions.

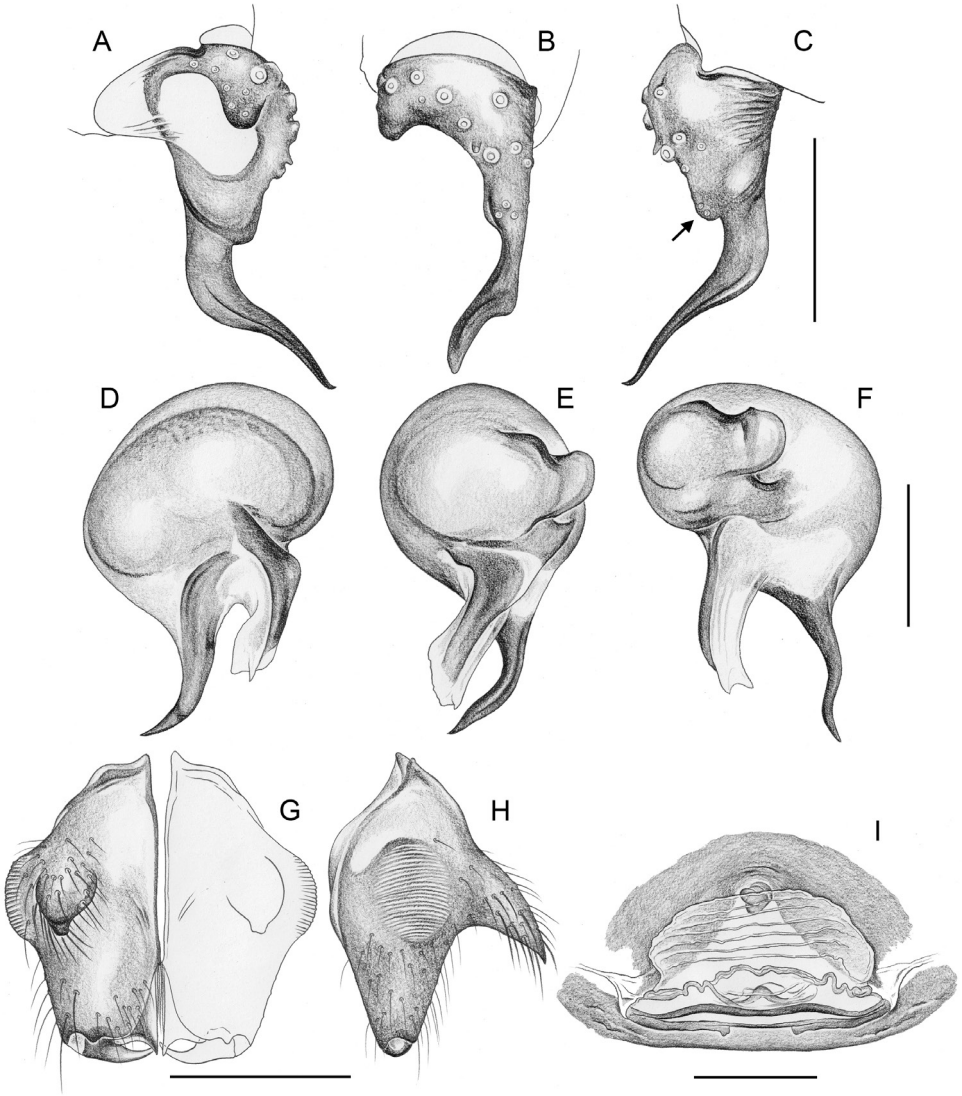

Figure 16. *Nerudia centaura* sp. nov.; non-type male and non-type female from Argentina, Catamarca, 20 km E Paso de San Francisco (ZFMK Ar 23891). A–C, left male palpal tarsus and procursus, prolateral, dorsal, and retrolateral views; arrow points at dorsal hump at basis of procursus. D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Scale lines: 0.2 mm.

Palps: As in Figure 15; coxa unmodified; trochanter with indistinct ventral projection; femur cylindrical, only slightly widened distally, proximally with indistinct retrolateral hump and prolateral stridulatory pick (modified hair); patella short; tibia globular; procursus (Fig. 16A–C) simple, in lateral view bent towards dorsal, in dorsal view bent towards prolateral, with pointed tip; genital bulb (Fig. 16D–F) large, with strong pointed ventral apophysis, embolus partly membranous, dorsally with distinct sclerite.

Legs: Without spines and curved hairs; vertical hairs in high densities on tibiae 1–2; retrolateral trichobothrium of tibia 1 at 68%; prolateral trichobothrium absent on tibia 1; tarsus 1 with six to seven pseudosegments, distally distinct.

Variation (male): Tibia 1 in 12 males (including holotype): 1.15–1.33 (mean 1.26).

Female: In general, similar to male (Fig. 1D) but with usual low density of vertical hairs on tibiae. Tibia 1 in 22 females: 1.07–1.48 (mean 1.28). Epigynum (Fig. 17A) anterior plate weakly protruding, with large, whitish posterior indentation; posterior plate large, simple. Internal genitalia (Figs 16I, 17B–E) with simple 'receptacle' (Fig. 17C) and unique tubular membranous structure in anterior position (possibly

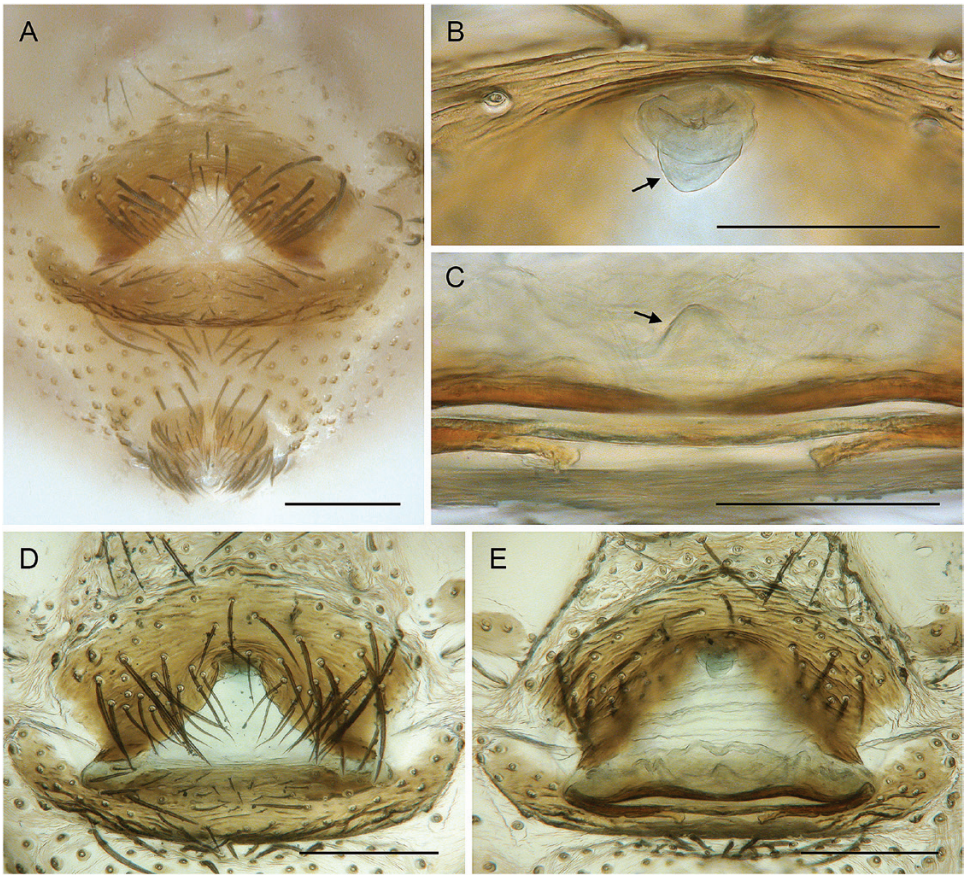

Figure 17. *Nerudia centaura* sp. nov.; non-type female from Argentina, Catamarca, 20 km E Paso de San Francisco (ZFMK Ar 23891). A, abdomen and epigynum, ventral view. B, anterior median tube-like structure in internal genitalia (arrow). C, posterior median structures in internal genitalia; arrow: 'receptacle'. D, E, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, D, E), 0.1 mm (B, C).

opening towards the outside, not towards the uterus externus).

Distribution: Known from two neighbouring localities in Argentina, Catamarca (Fig. 3).

Natural history: Both localities were similar, dominated by rocks and grasses [possibly *Calamagrostis crispa* (Rúgolo & Villav.) Govaerts; Julieta Carilla, pers. comm. August 2020] (Fig. 45C). The precipitation in this area is low (~150 mm mean annual precipitation) and largely limited to three months per year (~70% in December–February) (https://www.meteoblue.com/). We visited the locality at the end of the 'humid' season, in March, and often found humid patches of soil under large rocks, with considerable numbers of small, whitish collembolans. At the lower site (4180 m a.s.l.), this microhabitat seemed to contain little else than one species each of collembolans, pholcids, linyphiids and ants. At the higher site (4270–4450 m a.s.l.), the diversity seemed to be slightly higher, including, in addition, a species each of theridiids, filistatids and neopteran insects. At both localities, the pholcids were found sitting on the undersides of the rocks. They did not move and were thus easy to catch. Of the 36 females seen, only one had an egg-sac.

Precise climate data do not exist for these localities, but simulations of meteorological models (https://www.meteoblue.com/) suggest that temperatures fall below 0 °C almost every night of the year, with daily minima below –10 °C for seven months (April–October). In 2019, the temperatures repeatedly dropped below –20 °C in this period.

NERUDIA ROCIO HUBER SP. NOV.

(FIGS 19–21)

Zoobank registration: urn:lsid:zoobank.org:act:9245D3BD-222C-4C15-8D99-7D3CB98F848B.

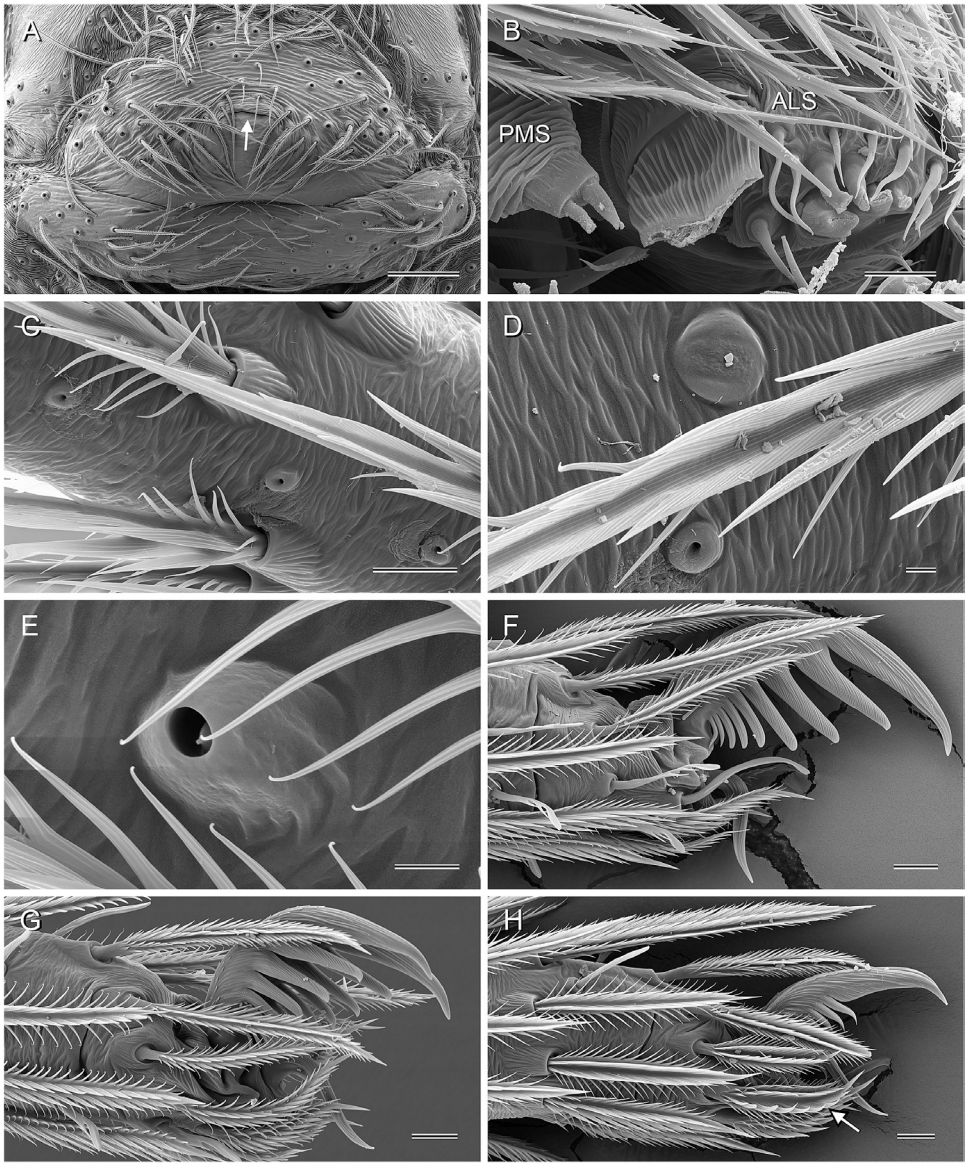

Figure 18. *Nerudia centaura* sp. nov.; female from Argentina, Catamarca, 20 km E Paso de San Francisco (ZFMK Arg216). A, epigynum, ventral view; arrow: possibly entry into tube-like internal structure. B, female ALS and PMS. C, putative glandular pores on female tarsus 3. D, glandular pore and slit sensillum(?) on female tarsus 4. E, tarsal organ on female tarsus 3. F, tip of female right tarsus 1, retrolateral view. G, tip of female right tarsus 3, retrolateral view. H, tip of female left tarsus 4, prolateral view; arrow: comb-hair. Scale lines: 100 µm (A), 10 µm (B, C, F–H), 2 µm (D, E).

Diagnosis: Easily distinguished from known congeners by shapes of procursus (Fig. 20A–C; distinctively widened tip); also by shape of embolus (Fig. 20D–F; distally strongly bent towards dorsal), by armature of male chelicerae (Fig. 20G, H; slender frontal apophyses, without stronger hairs), by epigynum and female internal genitalia (Figs 20I, 21; epigynal plate anteriorly narrow with strong transversal ridges; internally apparently without 'receptacle'), and by indistinct radial marks on carapace in males and females.

Type material: ARGENTINA – **San Juan**: • ♂ holotype; ~35 km W Las Flores; 30.3967° S, 69.5576° W; 2910 m a.s.l.; 6 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; *LABRE-Ar 588* • 2 ♂♂, 5 ♀♀, paratypes, + 2 juvs; same data as holotype; LABRE-Ar 536.

Other material examined: ARGENTINA – **San Juan**: • 2 ♀♀, in pure ethanol; same data as holotype; ZFMK Arg148 (one female abdomen transferred to 80% ethanol, ZFMK Ar 23893) • 1 ♀, in pure ethanol; same data as holotype; LABRE-Ar 543.

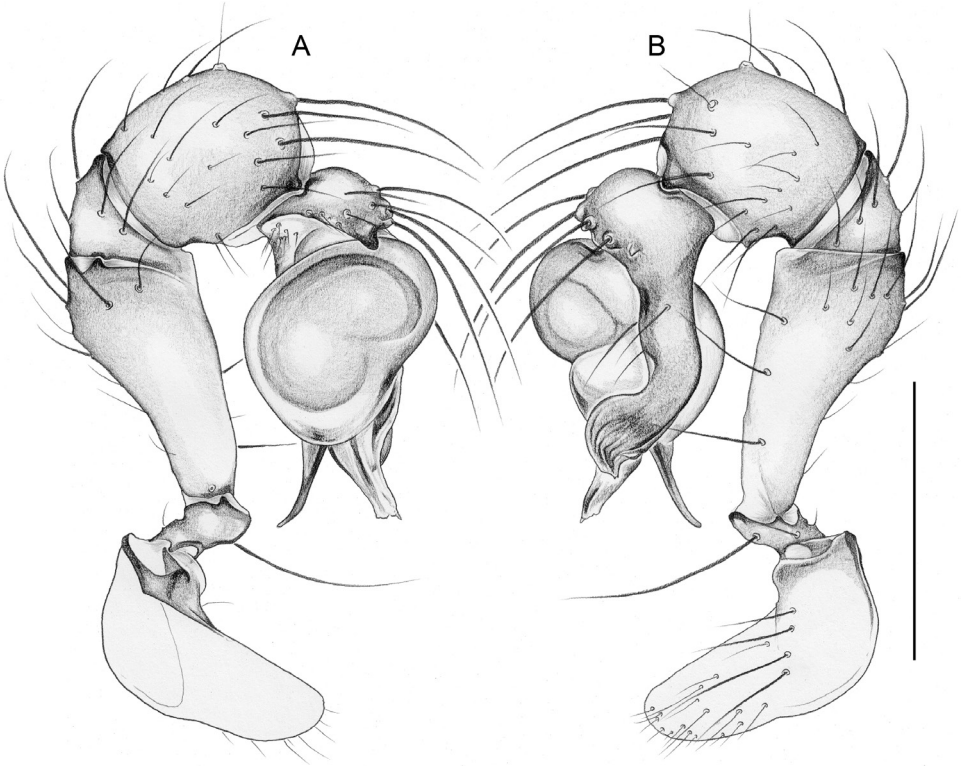

Figure 19. *Nerudia rocio* sp. nov.; holotype male from Argentina, San Juan, 35 km W Las Flores (*LABRE-Ar 588*). Left palp, prolateral (A) and retrolateral (B) views. Scale line: 0.3 mm.

Etymology: The species epithet *rocio* (rocío is Spanish for 'dew') is taken from Pablo Neruda's poem 'Poema 12'; noun in apposition.

Description

Male (holotype). Measurements: Total body length 1.60, carapace width 0.73. Distance PME–PME 80 µm; diameter PME 70 µm; distance PME–ALE 30 µm; distance AME–AME 20 µm; diameter AME 50 µm. Leg 1: 8.40 (2.25 + 0.30 + 2.50 + 2.55 + 0.80), tibia 2: 1.85, tibia 3: 1.45, tibia 4: 2.00; tibia 1 L/d: 31.

Colour (in ethanol): Carapace ochre-yellow with indistinct darker (ochre) median mark including ocular area and radial lateral marks; legs monochromous ochre-yellow, without dark rings; abdomen monochromous ochre-grey.

Body: Habitus similar to *N. poma* (cf. Fig. 1B). Ocular area barely raised. Carapace with indistinct thoracic groove. Clypeus unmodified, only rim slightly more sclerotized. Sternum wider than long (0.52/0.48), with pair of distinct anterior processes near coxae 1. Abdomen globular.

Chelicerae: As in Figure 20G, H; pair of pointed frontal apophyses directed forward and weakly curved downward; stridulatory files on pair of low lateral protrusions.

Palps: As in Figure 19; coxa unmodified; trochanter with indistinct ventral projection; femur cylindrical, slightly widened distally, proximally without retrolateral hump, with prolateral stridulatory pick (modified hair), femur length/width: 1.93; patella short; tibia globular, less strongly widened than in congeners (length/width: 1.17); procursus with distinctive distal widening and proximal dorsal ridge (Fig. 20A–C); genital bulb with simple ventral apophysis, embolus distally strongly bent towards dorsal (Fig. 20D–F).

Legs: Without spines and curved hairs; with vertical hairs in higher density on tibia 1, only proximally on prolateral and retrolateral sides (length ~20 µm; length of dorsal trichobothrium on tibia 1: ~80 µm); retrolateral trichobothrium of tibia 1 at 66%; prolateral trichobothrium absent on tibia 1; tarsus 1 with seven to eight pseudosegments, distally distinct.

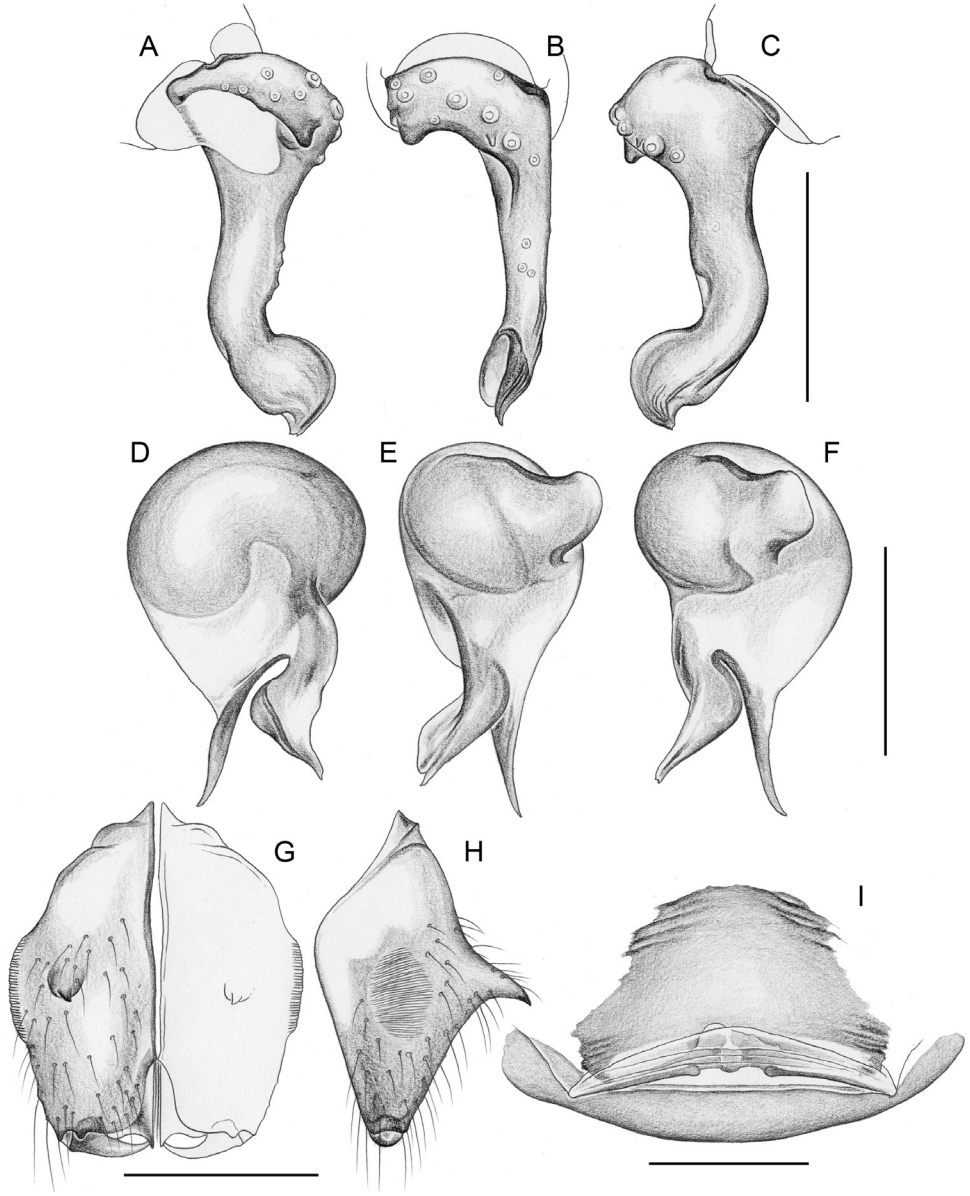

Figure 20. *Nerudia rocio* sp. nov.; holotype male (*LABRE-Ar 588*) and non-type female (ZFMK Ar 23893) from Argentina, San Juan, 35 km W Las Flores. A–C, left male palpal tarsus and procursus, prolateral, dorsal, and retrolateral views. D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Scale lines: 0.2 mm.

Variation (male): Tibia 1 in two other males: 2.10, 2.12.

Female (see Note below): In general, similar to male but sternum without pair of anterior humps. Tibia 1 in seven females: 1.61–2.00 (mean 1.80). Epigynum (Fig. 21A) anterior plate weakly protruding, anteriorly narrow with strong transversal ridges; posterior plate large, simple. Internal genitalia (Figs 20I, 21B–D) simple, apparently without or with small and indistinct median receptacle.

Distribution: Known from type locality only, in Argentina, San Juan (Fig. 3).

Natural history: The spiders were collected by turning large rocks on an arid hillside (Fig. 45D). They shared the microhabitat with another (undescribed) species

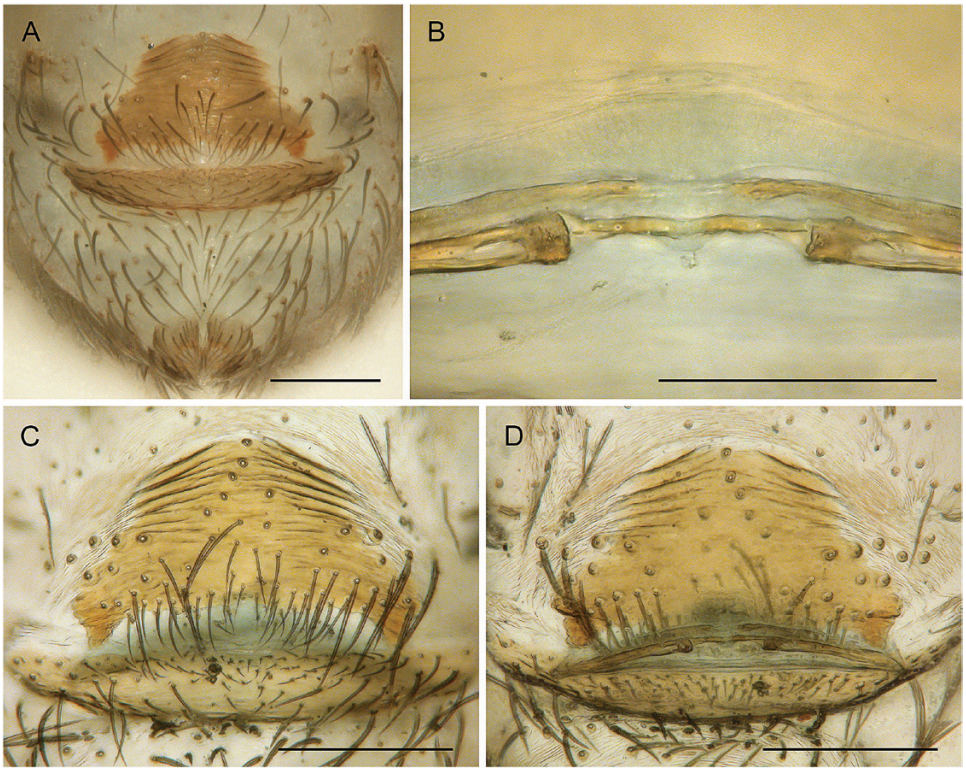

Figure 21. *Nerudia rocio* sp. nov.; non-type female from Argentina, San Juan, 35 km W Las Flores (ZFMK Ar 23893). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia. C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

of *Nerudia* (*N.* 'Arg23a'), of which only females were collected (see Note below). A third species collected at this locality (possibly *N. ola*; see Note below) was found closer to the river.

Note: Eighteen female specimens representing three putative species of *Nerudia* were collected at the type locality of this species. Eight specimens share with the male holotype (and male paratypes) the radial marks on the carapace and the relatively long legs. We thus consider these to be conspecific with the holotype. However, this needs confirmation, ideally by collecting males of the other putative species and by barcoding males and females. One female specimen shares with *N. ola* the shape of the epigynum and is thus tentatively assigned to that species. The remaining nine female specimens have a distinctive epigynum (Fig. 37A) and their separation from *N. rocio* and *N. ola* is also supported by *COI* (Fig. 2). They are thus considered to represent a separate undescribed species, *Nerudia* 'Arg23a' (see Putative further species at the end of the taxonomic section).

NERUDIA TRIGO HUBER SP. NOV.

(FIGS 1E, 22–24)

Zoobank registration: urn:lsid:zoobank.org:act:2D2F4A08-7C18-4ED6-8894-FE391F17E6C6.

Diagnosis: Easily distinguished from known congeners by male chelicerae (Fig. 22G, H; without frontal apophyses, with patches of strong hairs); also by shapes of procursus (Fig. 22A–C; similar to *N. guirnalda,* but without prolateral–ventral ridge proximally), by bulbal processes (Fig. 22D–F; ventral apophysis distally slender, curved towards ventral, same length as embolar process), and by epigynum and female internal genitalia (Figs 22I, 23; epigynal plate with short but wide posterior indentation, laterally strongly sclerotized; internal genitalia with indistinct 'receptacle'.

Type material: ARGENTINA – **Salta**: • ♂ holotype; between Alemanía and Cafayate; 25.7023° S, 65.7022° W; 1340 m a.s.l.; 23 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; *LABRE-Ar 589* • 1 ♂, 2 ♀♀, paratypes; same data as holotype; ZFMK Ar 23894.

Other material examined: ARGENTINA – **Salta**: • 10 ♀♀, 2 juvs, in pure ethanol (one female used for SEM; three female prosomata used for molecular study); same data as holotype; ZFMK Arg206 • 3 ♂♂; same data as holotype; LABRE-Ar 539 • 1 ♂; ~1 km SW Alemanía; 25.6300° S, 65.6180° W; 1210 m a.s.l.; 23 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.;

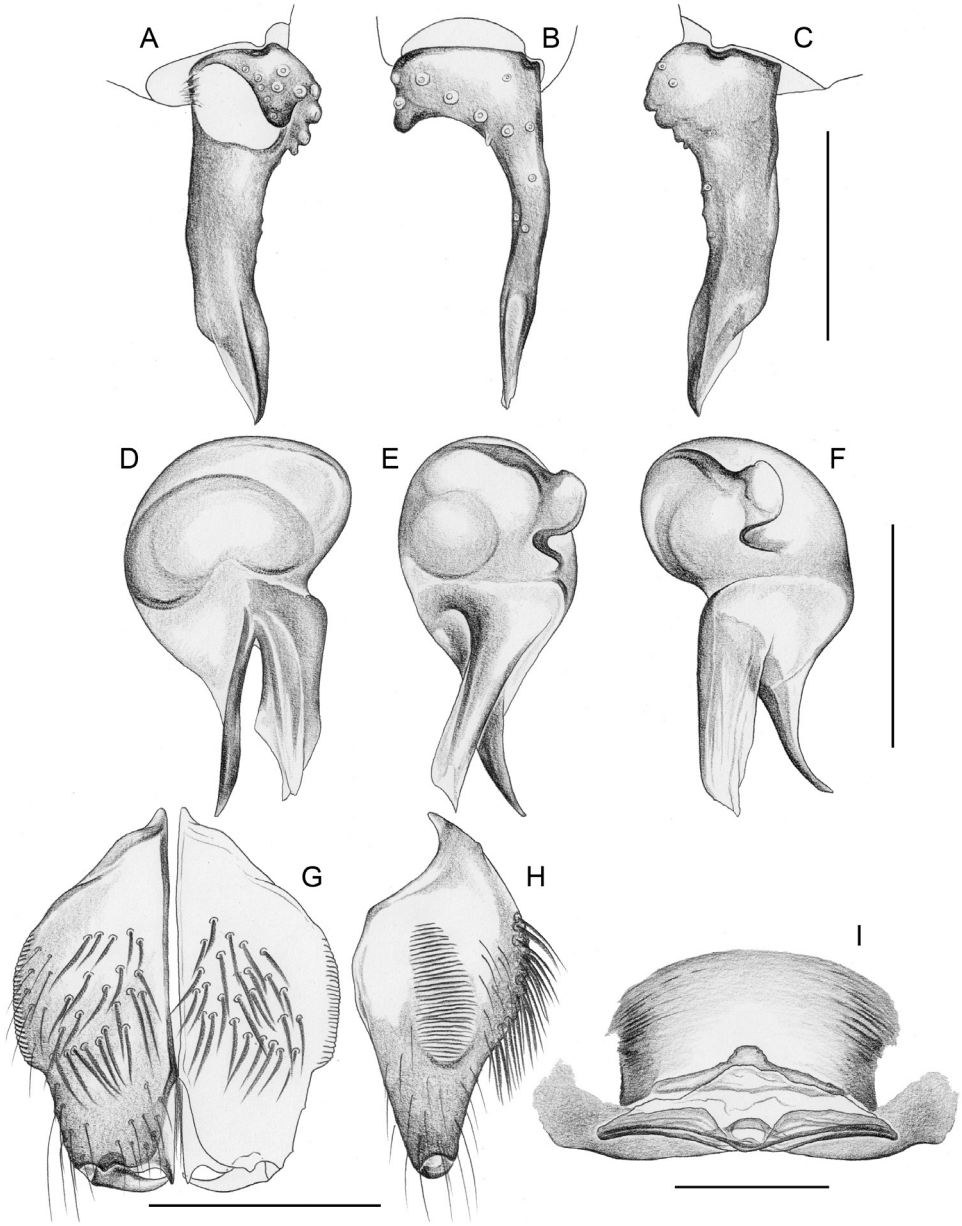

Figure 22. *Nerudia trigo* sp. nov.; paratype male and paratype female from Argentina, Salta, between Alemanía and Cafayate (ZFMK Ar 23894). A–C, left male palpal tarsus and procursus, prolateral, dorsal, and retrolateral views. D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Scale lines: 0.2 mm.

ZFMK Ar 23895 • 1 ♀, in pure ethanol; same data as preceding; ZFMK Arg202 • 2 ♀♀, 1 juv., in pure ethanol; same data as preceding; LABRE-Ar 550.

Etymology: The species epithet *trigo* (Spanish for 'wheat') is taken from Pablo Neruda's poem 'Cien sonetos de amor'; noun in apposition.

Description

Male (holotype). Measurements: Total body length 1.70, carapace width 0.77. Distance PME–PME 80 µm; diameter PME 65 µm; distance PME–ALE 20 µm; distance AME–AME 20 µm; diameter AME 50 µm. Leg 1: 7.50 (2.10 + 0.30 + 1.95 + 2.40 + 0.75), tibia 2: 1.80, tibia 3: 1.60, tibia 4: 2.10; tibia 1 L/d: 22.

Colour (in ethanol): Prosoma and legs mostly pale ochre-yellow; darker ochre Y-mark behind ocular area; legs without dark rings; abdomen monochromous pale grey.

Body: Habitus as in *N. poma* (cf. Fig. 1B). Ocular area barely raised. Carapace with indistinct thoracic

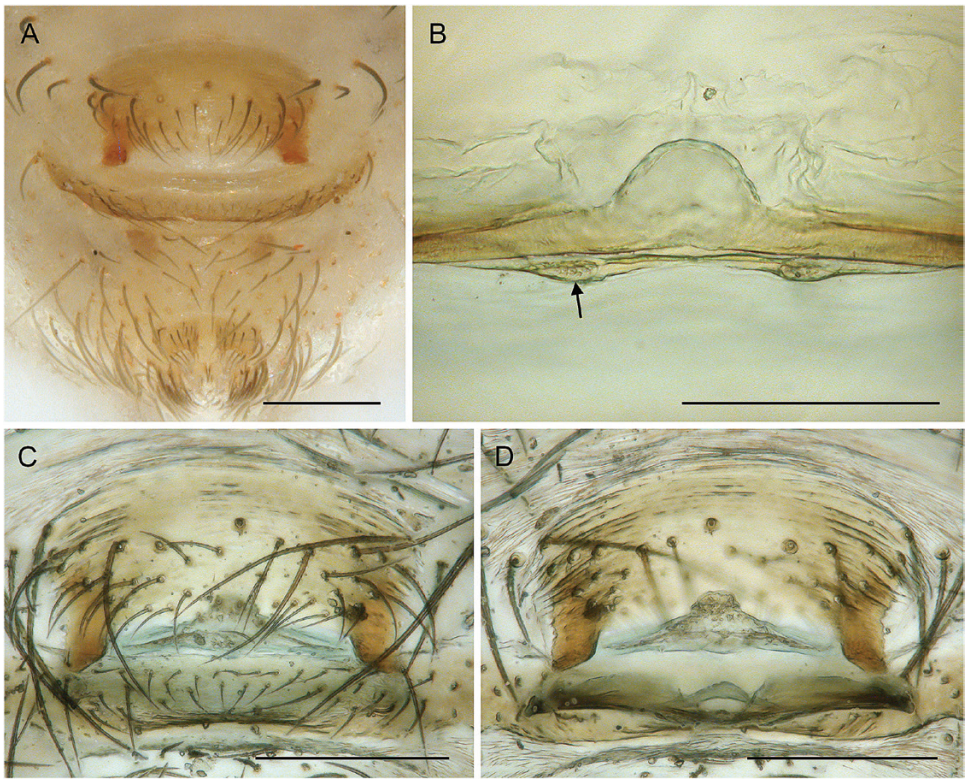

Figure 23. *Nerudia trigo* sp. nov.; paratype female from Argentina, Salta, between Alemanía and Cafayate (ZFMK Ar 23894). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia (arrow points at possible pore plate). C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

groove. Clypeus unmodified. Sternum wider than long (0.54/0.42), with pair of distinct anterior processes near coxae 1. Abdomen globular.

Chelicerae: As in Figure 22G, H; with large patch of strong hairs on each side; without frontal processes; stridulatory files on low lateral protrusions.

Palps: Similar to *N. colina* (cf. Fig. 4); proximal segments apparently identical to *N. colina*; femur length/width 2.08; tibia length/width 1.10; procursus simple, in lateral view slightly directed towards dorsal, similar to *N. colina* but without subdistal ventral sclerite and slightly wider (Fig. 22A–C); genital bulb similar to *N. colina* but ventral apophysis less strongly curved towards ventral (Fig. 22D–F).

Legs: Without spines and curved hairs; apparently few vertical hairs; retrolateral trichobothrium of tibia 1 at 63%; prolateral trichobothrium absent on tibia 1; tarsus 1 with seven to eight pseudosegments, distally distinct.

Variation (male): Tibia 1 in three other males from type locality: 1.71, 1.72, 1.83. The male from 1 km SW Alemanía has apparently identical chelicerae and pedipalps but is smaller: tibia 1: 1.40; carapace width: 0.63; chelicerae maximum width: 240 µm.

Female: In general, similar to male (Fig. 1E) but sternum without pair of anterior humps. Tibia 1 in four females: 1.46, 1.47, 1.65, 1.85. Epigynum (Fig. 23) anterior plate roughly rectangular, weakly protruding, with short but wide posterior indentation; posterior plate large, simple. Internal genitalia (Figs 22I, 23B–D) simple, with indistinct 'receptacle'.

Distribution: Known only from two neighbouring localities in Salta, Argentina (Fig. 3).

Natural history: At the type locality, the spiders were found in small cavities or shelters composed of large rocks in a dry ravine. They were sitting relatively exposed on the ceiling of the cavities and were easy to collect as they barely moved. Several females carried egg-sacs that were consistently reddish, flattened, and contained approximately 15–18 eggs. Near Alemanía, the single pair was found close together on the underside of a large rock. The spiders barely moved at disturbance. They shared the microhabitat with a tiny undescribed species of Modisiminae.

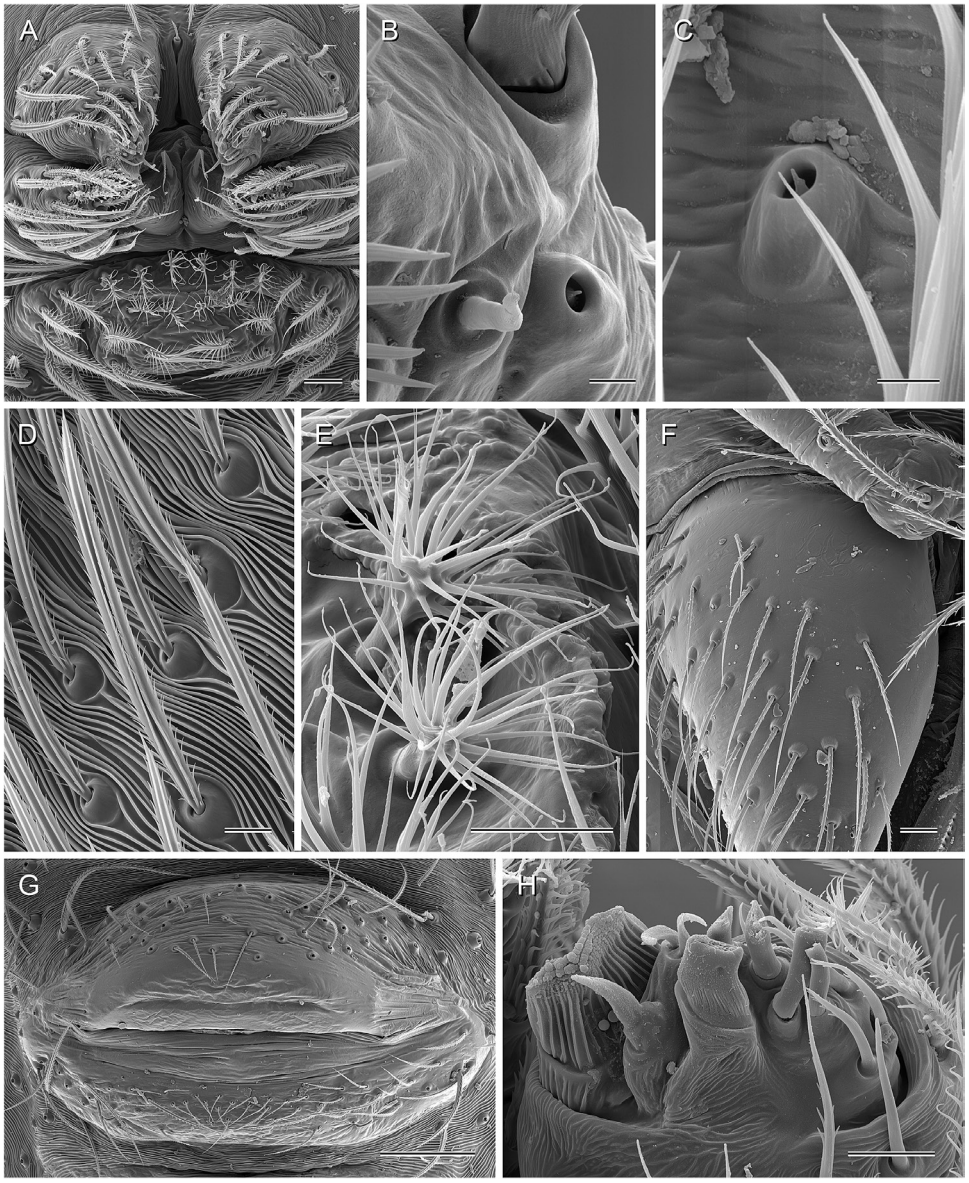

Figure 24. *Nerudia trigo* sp. nov.; female from Argentina, Salta, between Alemanía and Cafayate (ZFMK Arg206). A, female spinnerets. B, female palpal tarsal organ. C, tarsal organ on female right tarsus 4. D, 'regular' hairs on female abdomen. E, hairs on female anal cone. F, left female chelicera, lateral view (note absence of stridulatory file). G, epigynum, ventral view. H, female ALS. Scale lines: 20 µm (A, F), 2 µm (B, C), 10 µm (D, E, H), 100 µm (G).

NERUDIA GUIRNALDA HUBER **SP. NOV.**

(FIGS 1F, 25, 26)

Zoobank registration: urn:lsid:zoobank.org:act:7EF740D6-19C7-4A45-97BA-2FB853B8FB2F.

Diagnosis: Distinguished from most known congeners by shape of procursus (Fig. 25A–C; wide in lateral view, with prolateral–ventral ridge proximally), from the similar *N. trigo* by armature of male chelicerae (Fig. 25G, H; strong frontal apophyses pointing downward, with flattened tip; set with strong hairs), from some congeners also by bulbal processes (Fig. 25D–F; ventral apophysis slender, weakly curved, same length as embolus), and by epigynum and female internal genitalia (Figs 25I, 26; epigynal plate trapezoidal, medially light, much narrower than posterior plate; internal genitalia with posteriorly wide open receptacle; similar to *N. colina* and *N. trigo*).

Type material: ARGENTINA – **Catamarca**: • ♂ holotype; El Rodeo, trail to Cristo Redentor; 28.2229°

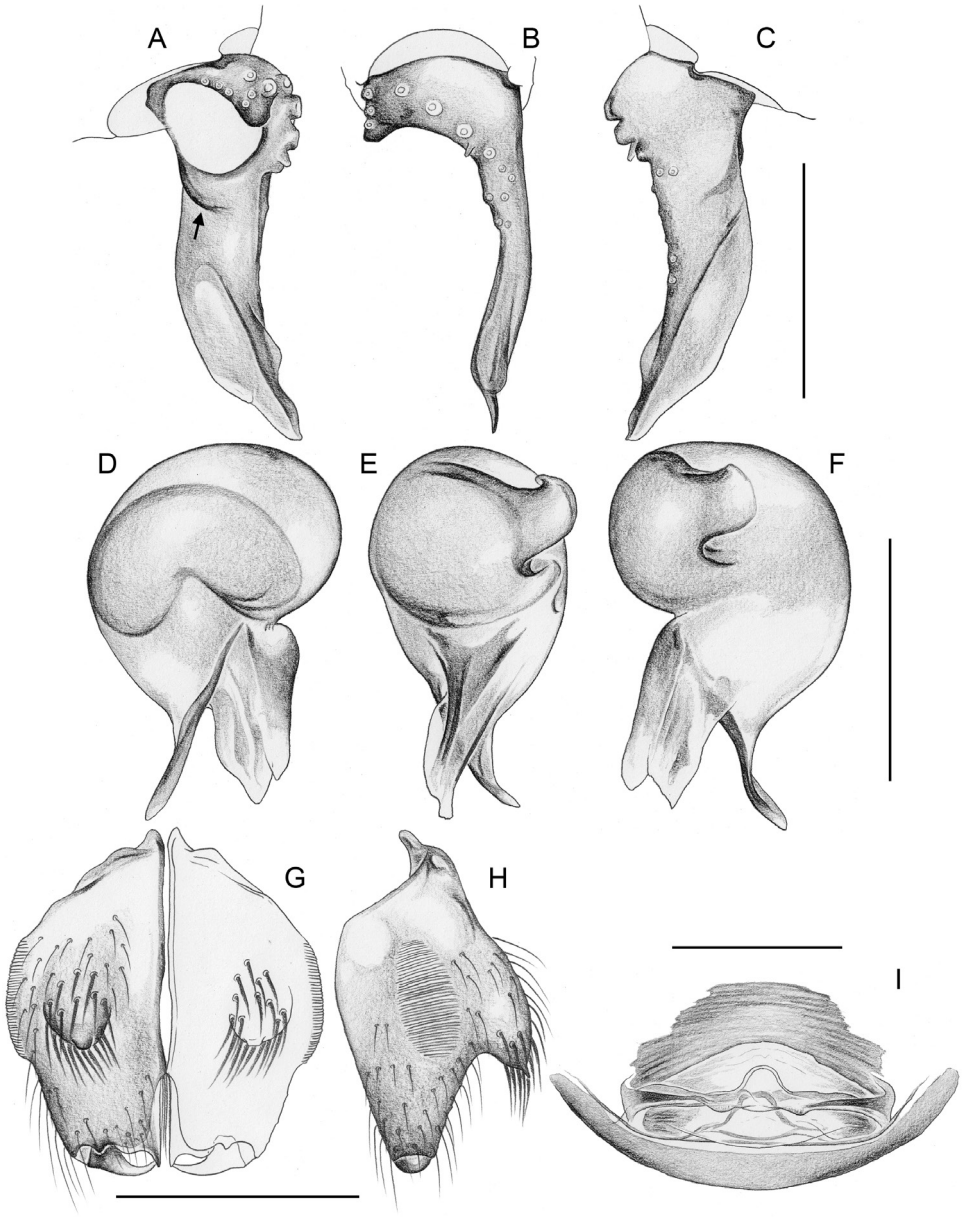

Figure 25. *Nerudia guirnalda* sp. nov.; paratype male and paratype female from Argentina, Catamarca, El Rodeo (ZFMK Ar 23896). A–C, left male palpal tarsus and procursus, prolateral, dorsal, and retrolateral views; arrow points at prolateral–ventral ridge. D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Scale lines: 0.2 mm.

S, 65.8677° W; 1460 m a.s.l.; 11 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; *LABRE-Ar 590* • 3 ♂♂, 4 ♀♀, paratypes; same data as holotype; ZFMK Ar 23896.

Other material examined: ARGENTINA – **Catamarca**: • 5 ♀♀, in pure ethanol (two prosomata used for molecular study); same data as holotype; ZFMK Arg166, 167 • 1 ♀; same data as holotype; LABRE-Ar 538 • 1 ♂; El Rodeo; Jan. 1957; M.E. Galiano leg.; MACN 20015 part • 2 ♂♂, 2 ♀♀, 1 juv.; Mutquin; ~28.32° S, 66.13° W; 2000 m a.s.l.; Jan. 1966; O. de Ferrariis leg.; MACN 20050 part.

Etymology: The species epithet *guirnalda* (Spanish for a 'garland') is taken from Pablo Neruda's poem 'Sed de ti'; noun in apposition.

Description

Male (holotype). Measurements: Total body length 1.40, carapace width 0.60. Distance PME–PME 70 µm;

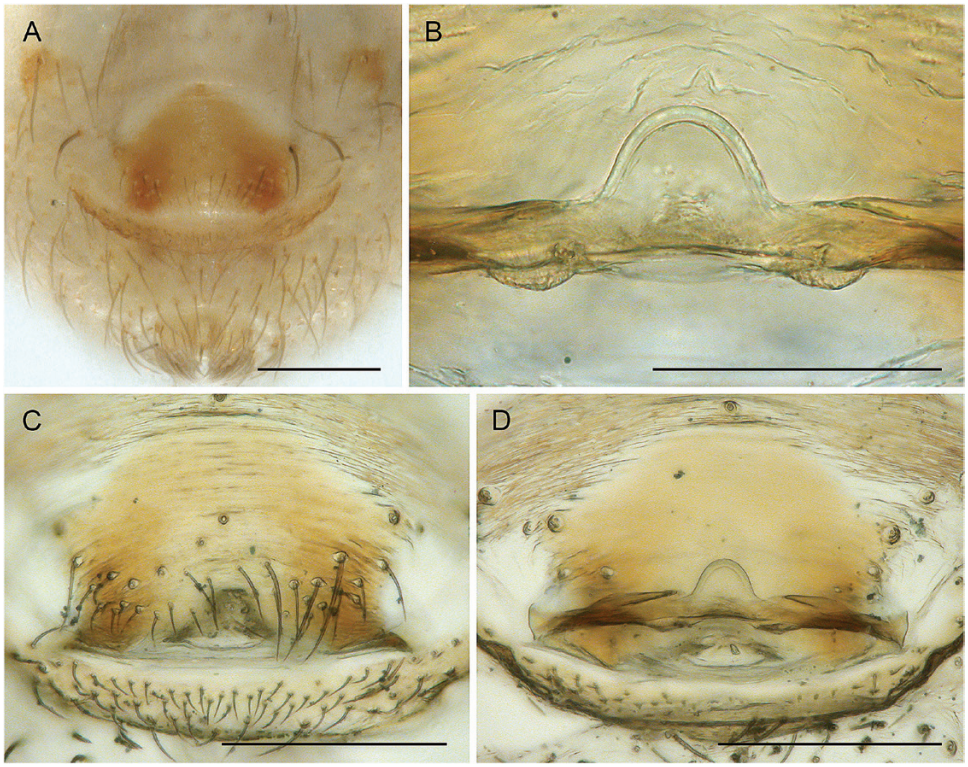

Figure 26. *Nerudia guirnalda* sp. nov.; paratype female from Argentina, Catamarca, El Rodeo (ZFMK Ar 23896). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia. C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

diameter PME 60 µm; distance PME–ALE 20 µm; distance AME–AME 15 µm; diameter AME 45 µm. Leg 1: 4.80 (1.37 + 0.23 + 1.23 + 1.40 + 0.57), tibia 2: 1.03, tibia 3: 0.87, tibia 4: 1.27; tibia 1 L/d: 18.

Colour (in ethanol): Prosoma and legs pale ochre-yellow; with indistinct Y-mark on carapace; legs without dark rings; abdomen ochre-yellow to light grey, with indistinct internal marks.

Body: Habitus as in Figure 1F. Ocular area barely raised. Carapace with indistinct thoracic groove. Clypeus unmodified, only at rim slightly sclerotized. Sternum wider than long (0.44/0.38), with pair of low anterior processes near coxae 1. Abdomen globular.

Chelicerae: As in Figure 25G, H; with pair of short frontal apophyses pointing downward, with flattened tip, i.e. wide in frontal view, pointed in lateral view; set with strong hairs; stridulatory files on pair of low lateral protrusions.

Palps: In general, similar to *N. colina* (cf. Fig. 4); coxa unmodified; trochanter with indistinct ventral projection; femur cylindrical, slightly widened distally, proximally with indistinct retrolateral hump and prolateral stridulatory pick (modified hair), femur length/width: 1.81; patella short; tibia globular (length/width: 1.05); procursus simple (Fig. 25A–C), wide but mostly semi-transparent in lateral view, with prolateral–ventral ridge proximally; genital bulb (Fig. 25D–F) with weakly curved ventral apophysis, embolus partly membranous.

Legs: Without spines and curved hairs; with vertical hairs in two rows (prolateral, retrolateral) proximally on tibia 1 only; retrolateral trichobothrium of tibia 1 at 62%; prolateral trichobothrium absent on tibia 1; tarsus 1 with ~six pseudosegments, distally distinct.

Variation (male): Tibia 1 in six males (including holotype): 1.22–1.47 (mean 1.33).

Female: In general, similar to male but sternum without pair of anterior humps. Tibia 1 in ten females: 1.08–1.33 (mean 1.22). Epigynum (Fig. 26A) anterior plate weakly protruding, trapezoidal, medially light; posterior plate wide but short. Internal genitalia (Figs 25I, 26B–D) with posteriorly wide open receptacle.

Distribution: Known from two localities in the Cerro el Manchao region in Catamarca, Argentina (Fig. 3).

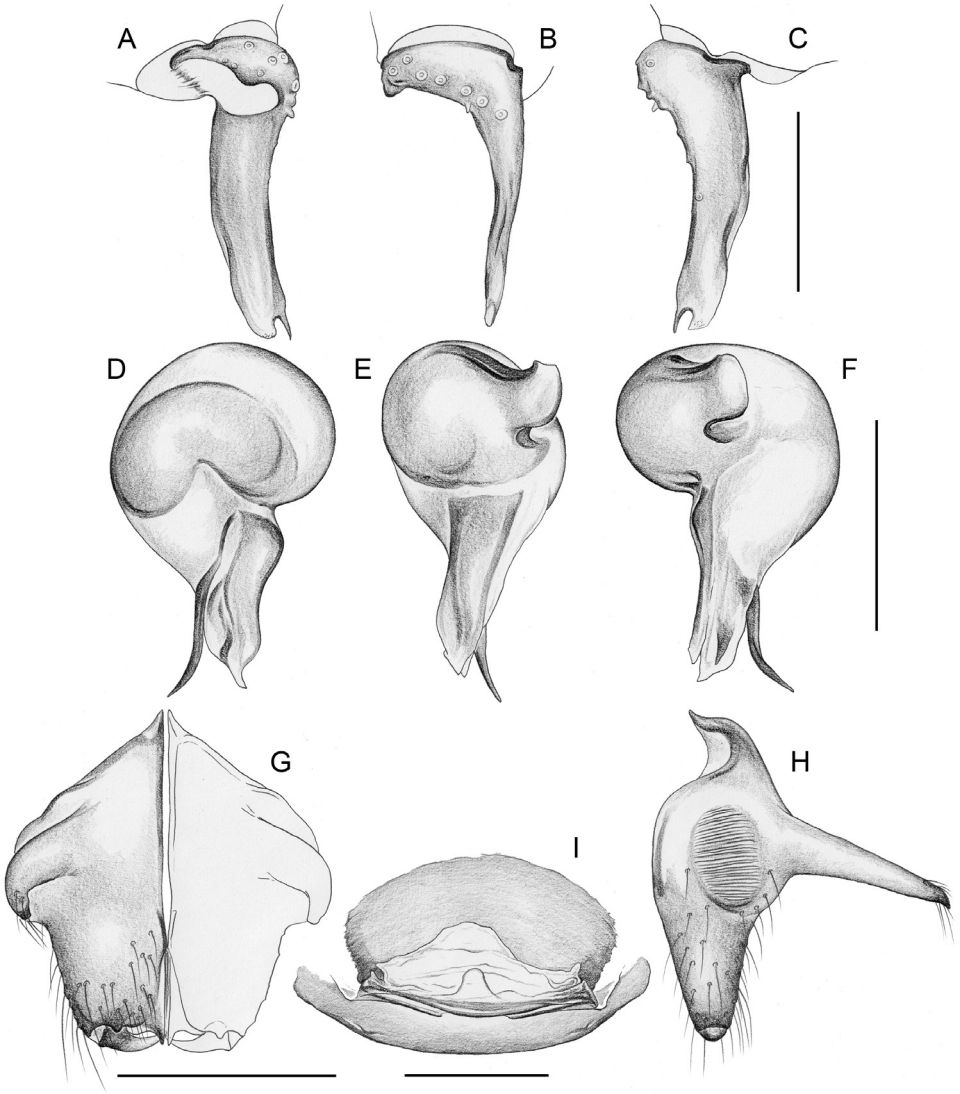

Figure 27. *Nerudia ola* sp. nov.; paratype male and paratype female from Argentina, San Juan, San Agustín de Valle Fértil (ZFMK Ar 23897). A–C, left male palpal tarsus and procursus, prolateral, dorsal, and retrolateral views. D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Scale lines: 0.2 mm.

Natural history: At the type locality the spiders were found by turning rocks along the trail in low forest.

NERUDIA OLA HUBER SP. NOV.

(FIGS 1G, 27–30)

Zoobank registration: urn:lsid:zoobank.org:act:BF76CDC2-9B0C-4B69-8DB9-DE6925D99DD4.

Nerudia Mich20: Eberle *et al.*, 2018 (molecular data: 28S). Huber *et al.*, 2018: fig. 2.

Diagnosis: Easily distinguished from known congeners by armature of male chelicerae (Fig. 27G, H; distinctive pair of long frontal apophyses) and by shape of procursus (Fig. 27A–C; with bifid tip consisting of slender dorsal process and wider ventral membranous part); also by bulbal processes (Fig. 27D–F; ventral apophysis distally slender, curved towards ventral, same length as embolus) and by epigynum and female internal genitalia (Figs 27I, 28; epigynal plate without posterior indentation; internal genitalia simple, with barely visible transparent 'receptacle').

Type material: ARGENTINA – **San Juan**: • ♂ holotype; San Agustín de Valle Fértil; 30.6366° S, 67.4863° W; 880 m a.s.l.; under rocks near river; 5 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; *LABRE-Ar*

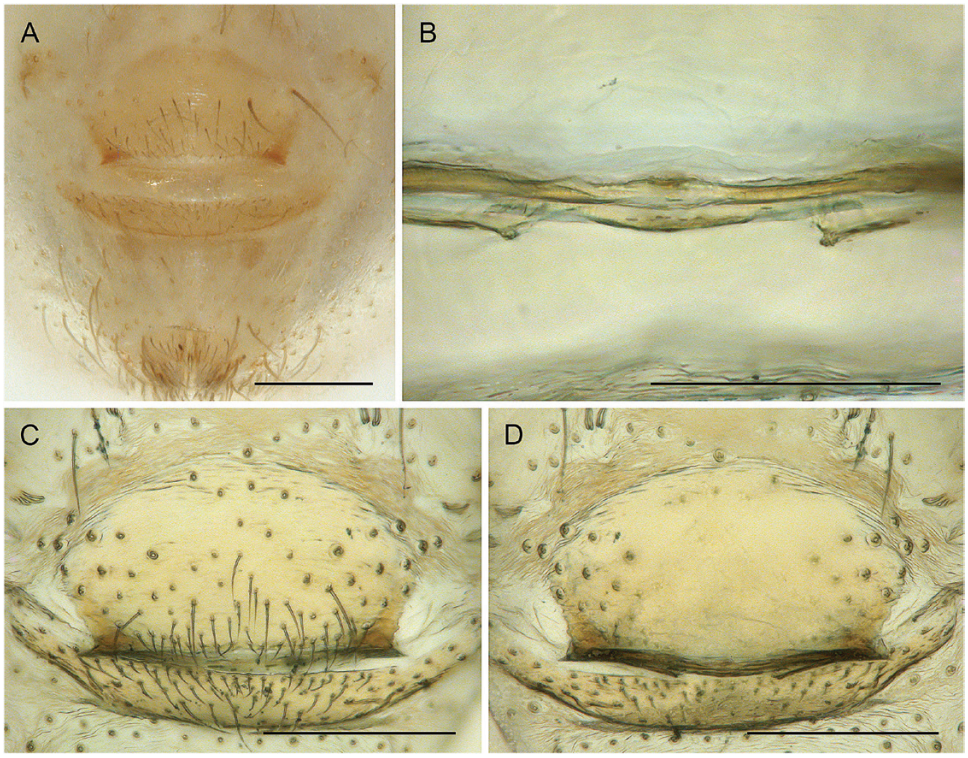

Figure 28. *Nerudia ola* sp. nov.; paratype female from Argentina, San Juan, San Agustín de Valle Fértil (ZFMK Ar 23897). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia. C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

591 • 5 ♂♂, 2 ♀♀, paratypes; same data as holotype; ZFMK Ar 23897.

Other material examined: ARGENTINA – **San Juan**: • 12 ♂♂, 8 ♀♀, 5 juvs, in pure ethanol (two males and two females used for SEM; three female prosomata used for molecular study); same data as holotype; ZFMK Arg141 • 9 ♀♀, 3 juvs; same data as holotype; LABRE-Ar 560 • 1 ♂, in pure ethanol; same locality as holotype; 22 Jan. 2012; J. M. A. Navarro leg.; ZFMK Mich20 • 3 ♂♂, 2 ♀♀; ~7.5 km S Astica; 31.0223° S, 67.2976° W; 865 m a.s.l.; under rocks near river; 4 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23898 • 1 ♂, 3 ♀♀, 5 juvs, in pure ethanol; same data as preceding; ZFMK Arg135, 136 • 1 ♂, 1 ♀; same data as preceding; LABRE-Ar 528 • 5 ♂♂, 7 ♀♀, 1 juv., in pure ethanol; same data as preceding; LABRE-Ar 529, 547, 548, 552 • 1 ♂, 1 ♀; Parque Provincial Ischigualasto; 30.1839° S, 67.9026° W; 1425 m a.s.l.; under rocks; 5 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23899 • 2 ♀♀, in pure ethanol; same data as preceding; ZFMK Arg144 • 1 ♀, 2 juvs; same data as preceding; LABRE-Ar 531 • 1 ♀ with eggs, in pure ethanol; same data as preceding; LABRE-Ar 554 • 1 ♂; Parque Provincial Ischigualasto; 30.1821° S, 67.9010° W; 1425 m a.s.l.; in dry riverbed, hand collecting at night; 19 Dec. 2018; M. Izquierdo, F. Bollatti, A. Albin, and C. Mattoni leg.; LABRE-Ar 445 • 1 ♂; same data as preceding; LABRE-Ar 444, prep. code MAI-4709 • 1 ♀; Baldecitos; 30.2232° S, 67.6942° W; 1255 m a.s.l.; stone wall of buildings, hand collecting at night; 21 Dec. 2018; M. Izquierdo, F. Bollatti, A. Albin, and C. Mattoni leg.; LABRE-Ar 448, prep. code MAI-4710.

Assigned tentatively (see Male variation below): **La Rioja**: • 1 ♂, 1 ♀; Cuesta de Miranda, 'site 1'; 29.3511° S, 67.7924° W); 1960 m a.s.l.; under rocks; 8 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23900 • 2 ♀♀, in pure ethanol; same data as preceding; ZFMK Arg152 • 2 ♂♂, 1 ♀; Cuesta de Miranda, 'site 2'; 29.3468° S, 67.7205° W; 1660 m a.s.l.; under rocks; 8 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23901 • 2 ♀♀, in pure ethanol; same data as preceding; ZFMK Arg154 • 1 ♂, 2 ♀♀; same data as preceding; LABRE-Ar 533 • 1 ♀, in pure ethanol; same data as preceding; LABRE-Ar 553 • 1 ♀; between Chilecito and Famatina; 29.0027° S, 67.4855° W; 1300 m a.s.l.; 9 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23902 • 1 ♀; same data as preceding; LABRE-Ar 541 • 1 ♀ with eggs, in pure ethanol; same data as preceding; LABRE-Ar

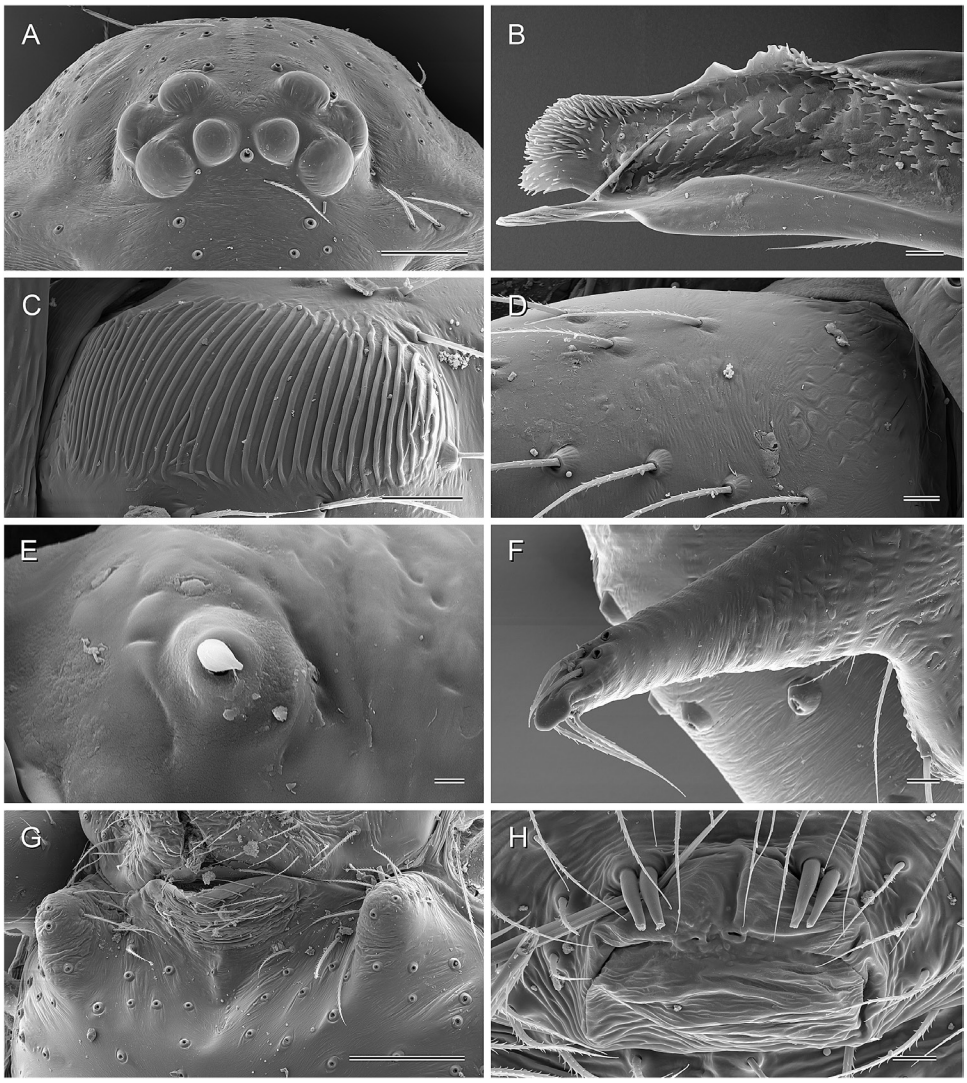

Figure 29. *Nerudia ola* sp. nov.; male and female from Argentina, San Juan, San Agustín de Valle Fértil (ZFMK Arg141). A, female prosoma, frontal view. B, left procursus tip, prolateral-dorsal view. C, stridulatory file on male chelicera. D, lateral view of female chelicera, showing absence of stridulatory file. E, stridulatory pick (modified hair) on male palpal femur. F, right male cheliceral apophysis, frontal view. G, processes on male sternum. H, male gonopore. Scale lines: 100 µm (A, G), 10 µm (B, D, F, H), 20 µm (C), 2 µm (E).

546 • 4 ♂♂, 2 ♀♀; SE Aimogasta, 'site 2'; 28.9015° S, 66.6538° W; 755 m a.s.l.; under rocks; 10 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23903 • 3 ♀♀, in pure ethanol; same data as preceding; ZFMK Arg162 • 1 ♂, 1 ♀, 1 juv.; same data as preceding; LABRE-Ar 532 • 1 ♀, in pure ethanol; same data as preceding; LABRE-Ar 567. **Catamarca**: • 2 ♂♂, 4 ♀♀; ~5 km NW Chumbicha, near Balneario El Caolín, 'site 2'; 28.8109° S, 66.2500° W; 640 m a.s.l.; steep rock field in forest; 28–29 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23904 • 7 ♀♀, 3 juvs, in pure ethanol (three female prosomata used for molecular study); same data as preceding; ZFMK Arg218 • 2 ♂♂, 6 ♀♀, 8 juvs, in pure ethanol; same data as preceding; LABRE-Ar 530, 544, 561, 563, 566 • 3 ♀♀ with eggs, in pure ethanol; same locality as preceding, 'site 1'; 28.8152° S, 66.2478° W; 605 m a.s.l.; M. A. Izquierdo and B. A. Huber leg.; LABRE-Ar 556 • 2 ♀♀, 8 juvs, in pure ethanol; same data as preceding; LABRE-Ar564 • 3 ♂♂, 2 ♀♀ (one male used for µ-CT study); ~10 km N Belén; 27.5641° S, 67.0058° W; 1380 m a.s.l.; in pile of stones; 25 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23905 • 5 ♀♀, 1 juv., in pure ethanol; same data as preceding; ZFMK Arg213 • 1 ♂, 7 ♀♀, 1 juv., in pure ethanol; same data as preceding; LABRE-Ar 545 • 11 ♂♂, 2 ♀♀ (three males and one female used for µ-CT

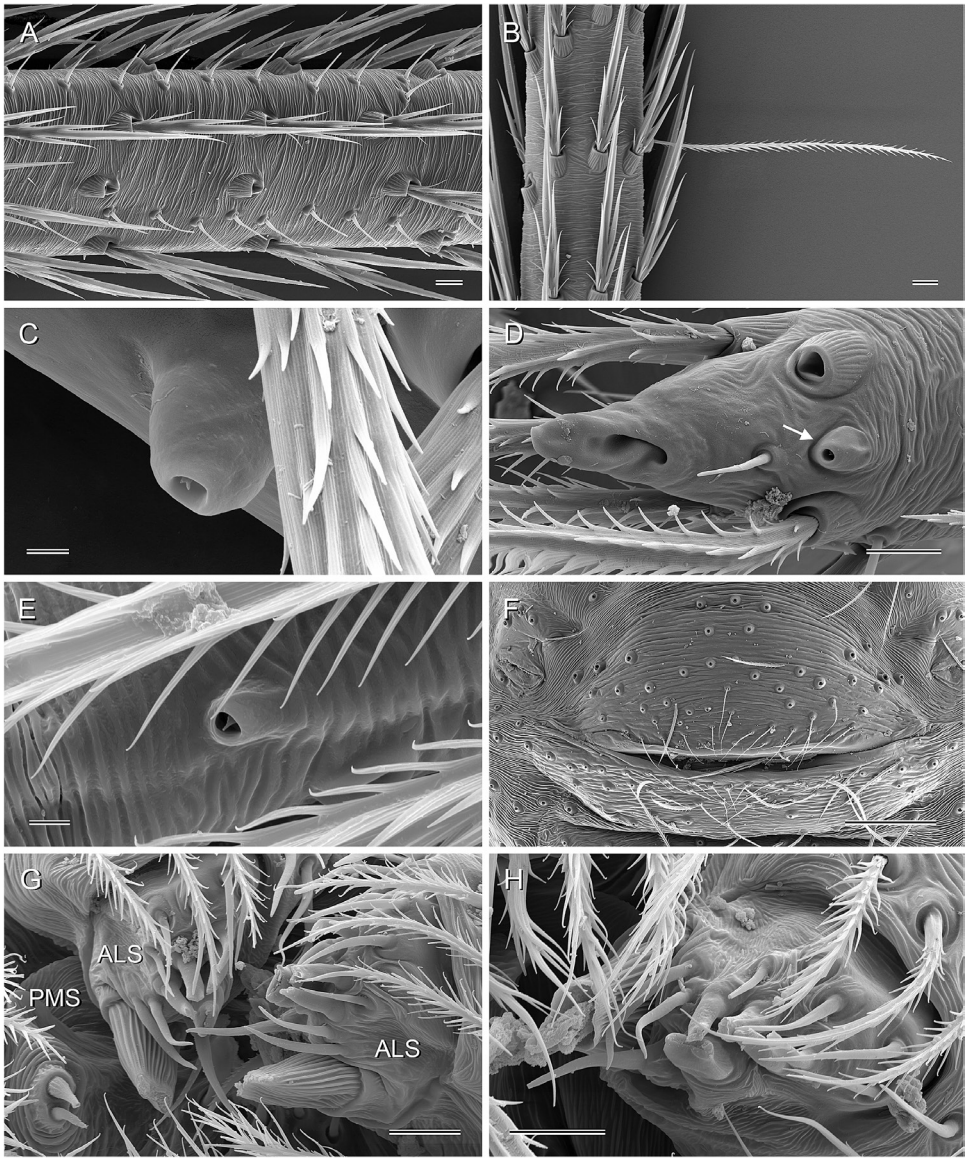

Figure 30. *Nerudia ola* sp. nov.; male and female from Argentina, San Juan, San Agustín de Valle Fértil (ZFMK Arg141). A, male right tibia 1, retrolateral view. B, male metatarsus 4 with trichobothrium. C, male palpal tarsal organ. D, tip of right female palp, dorsal view; arrow points at tarsal organ. E, tarsal organ on male tarsus 2. F, epigynum, ventral view. G, male ALS and PMS. H, female ALS. Scale lines: 10 µm (A, B, D, G, H), 2 µm (C, E), 100 µm (F).

study; two male abdomens used for karyotype study); near Nacimientos; 27.1559° S, 66.6925° W; 2120 m a.s.l.; under rocks on arid slope; 25 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23906 • 4 ♀♀, 3 juvs, in pure ethanol; same data as preceding; ZFMK Arg211.

Assigned tentatively (only females available): ARGENTINA – **La Rioja**: • 2 ♀♀, 1 juv.; Cuesta de Miranda; 29°21' S, 67°43' W; 1700 m a.s.l.; Aug. 1994; M. Ramírez leg.; MACN Ar 20055 • 1 ♀, in pure ethanol; SE Aimogasta, 'site 1'; 28.8069° S, 66.6635° W; 915 m a.s.l.; under rocks; 10 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Arg161. **Catamarca**: • 1 ♀; ~14 km W Fiambalá; 27.6590° S, 67.7607° W; 2000 m a.s.l.; under rocks; 26 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23907 • 2 ♀♀; Chumbicha; 28.87° S, 66.23° W; 400 m a.s.l.; Aug. 1994; M.J. Ramírez leg.; MACN Ar 20012. **San Juan**: • 1 ♀; ~35 km W Las Flores; 30.3967° S, 69.5576° W; 2910 m a.s.l.; under rocks; 6 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23908.

Etymology: The species epithet *ola* (Spanish for 'wave') is taken from Pablo Neruda's poem 'Poema 12'; noun in apposition.

Description

Male (holotype). Measurements: Total body length 1.55, carapace width 0.66. Distance PME–PME 70 µm; diameter PME 65 µm; distance PME–ALE 30 µm; distance AME–AME 20 µm; diameter AME 40 µm. Leg 1: 5.64 (1.48 + 0.25 + 1.45 + 1.78 + 0.68), tibia 2: 1.18, tibia 3: 1.03, tibia 4: 1.53; tibia 1 L/d: 22.

Colour (in ethanol): Prosoma and legs mostly pale ochre-yellow; carapace with light brown Y-mark behind ocular area; sternum whitish; legs without dark rings; abdomen monochromous pale grey.

Body: Habitus as in Figure 1G. Ocular area barely raised. Carapace with indistinct thoracic groove. Clypeus unmodified (only rim slightly more sclerotized than in female). Sternum wider than long (0.46/0.38), with pair of distinct anterior processes near coxae 1. Abdomen globular.

Chelicerae: As in Figure 27G, H; with distinctive pair of long frontal apophyses, tips slightly bent downwards, obtuse in frontal view, with some ventral hairs directed downwards (Fig. 29F); distance between tips of apophyses: 280 µm; stridulatory files on distinct lateral protrusions (Fig. 29C).

Palps: In general, similar to *N. colina* (cf. Fig. 4) but femur slightly slenderer (length/width 2.27) and tibia slightly thicker (length/width 0.92); procursus simple, in lateral view slightly directed towards dorsal, with bifid tip consisting of slender dorsal process and wider ventral membranous process (Fig. 27A–C); genital bulb similar to *N. colina*, with slender ventral apophysis curved towards ventral (Fig. 27D–F).

Legs: Without spines and curved hairs; with vertical hairs in higher than usual density on proximal half of tibia 1 only, in two dorsal rows (Fig. 30A); retrolateral trichobothrium of tibia 1 at 58%; prolateral trichobothrium absent on tibia 1; tarsus 1 with six to seven pseudosegments, distally distinct.

Variation (male): Tibia 1 in 36 males (including holotype): 1.04–1.60 (mean 1.33). The distance between the tips of the cheliceral apophyses varies considerably (235–340 µm), but also within localities (e.g. Nacimientos: 240–310 µm). In northern specimens (from La Rioja and Catamarca), the cheliceral apophyses are slightly straighter and longer. This difference is minimal and some males appear intermediate. In specimens from Chumbicha, the procursus is slightly shorter. Abdomen variably dark. *COI* data indicate a deep split between northern and southern specimens, and a phylogenetic analysis of the molecular data (Supporting Information, Fig. S2) even questions the monophyly of the northern + southern clade. However, deep splits also occur among southern specimens and among northern specimens (Fig. 2). As a result, a preliminary ASAP analysis (Supporting Information, Figs S3, S4) favours the existence of up to seven 'species' (lowest scores of 2.0 and 3.0). What is here interpreted as one species may thus in fact be several more or less cryptic species.

Female: In general, similar to male but sternum without pair of anterior humps and tibia 1 with usual low number of short vertical hairs. Tibia 1 in 57 females: 0.97–1.55 (mean 1.25). Epigynum (Figs 28A, 30F) anterior plate weakly protruding, without posterior indentation; posterior plate large, simple. Internal genitalia (Figs 27I, 28B–D) simple, with barely visible transparent receptacle. No difference could be seen between northern and southern specimens.

Distribution: Widely distributed in Argentina, Provinces San Juan, La Rioja and Catamarca (Fig. 3). Note that specimens from La Rioja and Catamarca are assigned tentatively.

Natural history: All specimens were found by turning rocks. The spiders started to run rapidly when disturbed but then stopped suddenly and did barely ever drop from the rock. They were found in a variety of habitats, ranging from a relatively humid block field in a low forest at 640 m a.s.l. near Chumbicha to an exposed arid hill with dry ravines at 2120 m a.s.l. near Nacimientos. At Valle Fértil, the spiders seemed to have a patchy distribution, with up to eight adult specimens on a single large rock in suitable places (with some shade and leaf litter among the rocks). They shared the microhabitat with a superficially similar species of *Metagonia* Simon, 1893 (*M.* 'MACN79'; undescribed; Valle Fértil; Chumbicha), with *Gertschiola macrostyla* (Astica; Valle Fértil; Ischigualasto; SE Aimogasta 'site 2'), with *Chibchea araona* Huber, 2000 (?) (Cuesta de Miranda, both sites) and, in at least one case, with another species of *Nerudia* (*N. poma*; Chumbicha).

NERUDIA NONO HUBER SP. NOV.

(FIGS 1H, 31, 32)

Zoobank registration: urn:lsid:zoobank.org:act:D31A81F7-8F7F-4DCA-BE7E-6EEAFFE52FBF.

Diagnosis: Distinguished from known congeners by shapes of procursus (Fig. 31A–C; wide in lateral view, narrow in dorsal view, with small prolateral flap distally) and by armature of male chelicerae (Fig. 31G, H; frontal apophyses directed towards frontal, with pointed tips), from some congeners also by bulbal processes (Fig. 31D–F; ventral apophysis

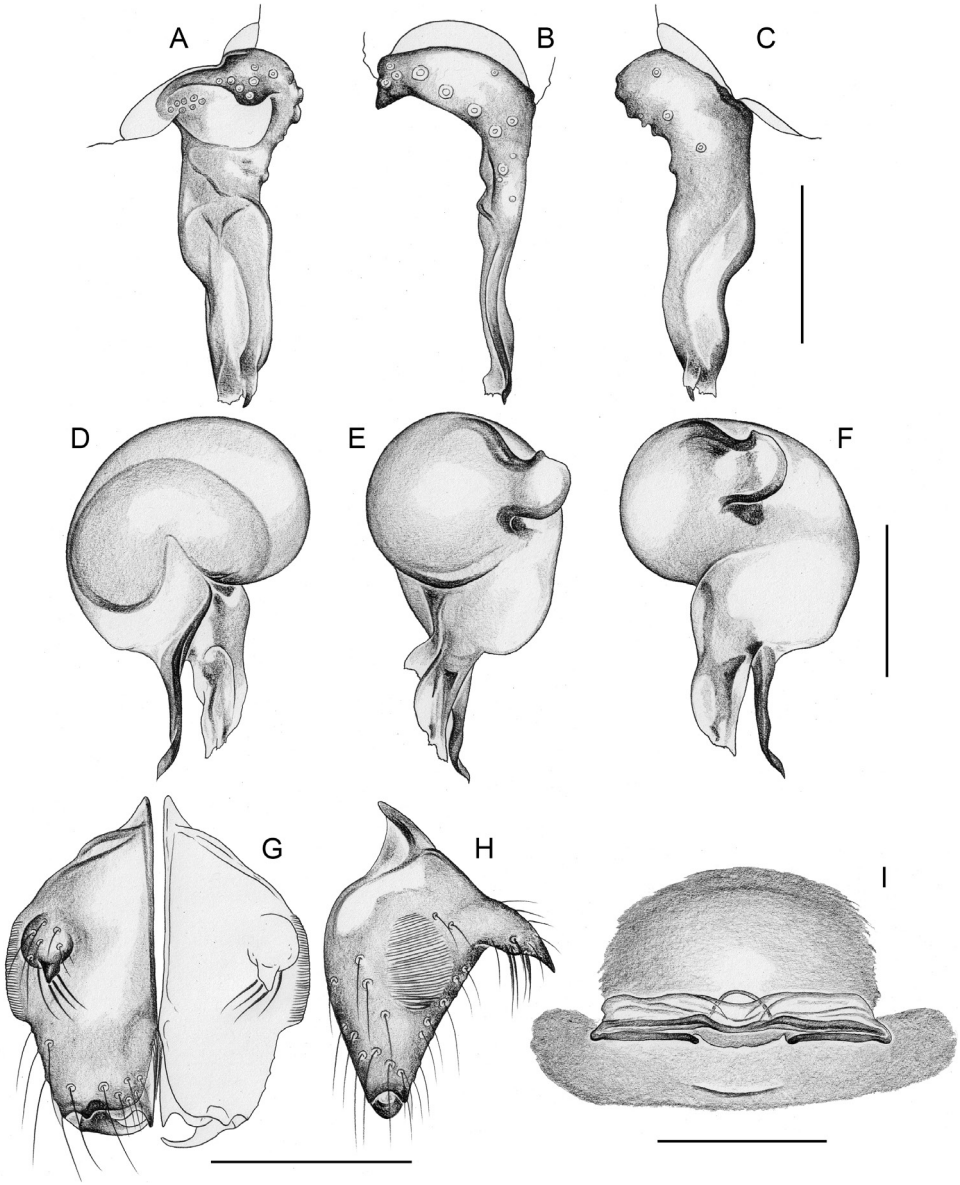

Figure 31. *Nerudia nono* sp. nov.; paratypes from Argentina, Córdoba, 5 km E Nono (♂ LABRE AR525; ♀ ZFMK Ar 23910). A–C, left male palpal tarsus and procursus, prolateral, dorsal, and retrolateral views. D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Scale lines: 0.2 mm.

distally slender, curved towards ventral, slightly longer than embolus), and by epigynum and female internal genitalia (Figs 31I, 32; main epigynal plate semi-circular, with almost straight posterior margin; internal genitalia with indistinct semi-circular receptacle).

Type material: ARGENTINA – **Córdoba**: • ♂ holotype; ~5 km E Nono; 31.7982° S, 64.9515° W; 995 m a.s.l.; 20 Feb. 2021; M. Izquierdo, F. Cargnelutti, F. Bollatti & G. Boaglio leg.; *LABRE-Ar 592* • 3 ♂♂, paratypes; same data as holotype; LABRE AR525 • 1 ♂, paratype; same data as holotype; ZFMK Ar 23909 • 1 ♀, paratype; same data as holotype; LABRE AR534 • 3 ♀♀, paratypes; same locality as holotype; 2 Mar 2019; B.A. Huber & M.A. Izquierdo leg.; ZFMK Ar 23910.

Other material examined: ARGENTINA – **Córdoba**: • 5 ♀♀, 7 juvs, in pure ethanol; same locality as holotype; 2 Mar 2019; B.A. Huber & M.A. Izquierdo leg.; ZFMK Arg124 • 1 ♂, 1 ♀; same locality as holotype; 5 Jan. 2022; M. Izquierdo, F. Cargnelutti, G. Boaglio leg.;

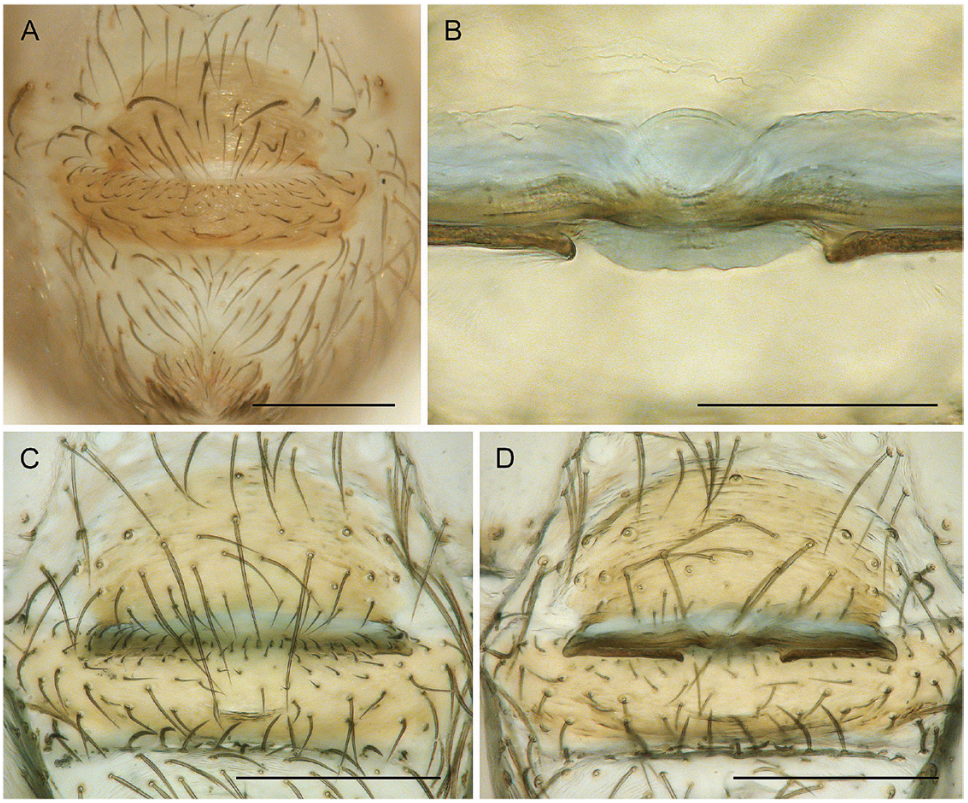

Figure 32. *Nerudia nono* sp. nov.; paratype female from Argentina, Córdoba, 5 km E Nono (ZFMK Ar 23910). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia. C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

LABRE-Ar 593 • 6 ♀♀; same data as preceding; LABRE-Ar 595 • 2 ♂♂, 1 ♀, 1 juv.; same locality as holotype; 20 Feb. 2021; M. Izquierdo, F. Cargnelutti, F. Bollatti, G. Boaglio, leg.; LABRE-Ar 594 • 2 ♂♂, in Karnovsky (MAI-4780); same data as preceding, LABRE-Ar 568 • 1 ♂, in Karnovsky (MAI-4771); same data as preceding; LABRE-Ar 569 • 6 ♀♀, in Karnovsky (MAI-4781); same data as preceding; LABRE-Ar 570.

Etymology: The species epithet is derived from the type locality Nono in Córdoba, Argentina; noun in apposition.

Description

Male (holotype. Measurements: Total body length 1.70, carapace width 0.70. Distance PME-PME 90 µm; diameter PME 60 µm; distance PME–ALE 35 µm; distance AME–AME 20 µm; diameter AME 50 µm. Leg 1: 5.20 (1.40 + 0.25 + 1.40 + 1.55 + 0.60), tibia 2: 1.15, tibia 3: 0.95, tibia 4: 1.40; tibia 1 L/d: 20.

Colour (in ethanol): Prosoma and legs mostly pale ochre-grey; carapace with large median brown mark including ocular area, not reaching posterior margin of carapace; sternum whitish; legs without dark rings; abdomen monochromous greenish-grey.

Body: Habitus as in Figure 1H. Ocular area barely raised. Carapace with indistinct thoracic groove. Clypeus more sclerotized than in female and slightly bulging. Sternum wider than long (0.50/0.40), with pair of distinct anterior processes near coxae 1. Abdomen globular.

Chelicerae: As in Figure 31G, H; pair of frontal apophyses directed forward, with flattened tips; stridulatory files on pair of lateral protrusions, ridges barely visible in dissecting microscope.

Palps: In general, similar to *N. colina* (cf. Fig. 4); coxa unmodified; trochanter with indistinct ventral projection; femur cylindrical, only slightly widened distally, proximally with indistinct retrolateral hump and prolateral stridulatory pick (modified hair); patella short, dorsally more bulging than in *N. colina*; tibia globular; procursus wide in lateral view, narrow in dorsal view, distally with small apophysis and

prolateral flap (Fig. 31A–C); genital bulb with ventral apophysis distally slender, curved towards ventral, embolus partly membranous (Fig. 31D–F).

Legs: Without spines and curved hairs; few vertical hairs; retrolateral trichobothrium of tibia 1 at 59%; prolateral trichobothrium absent on tibia 1; tarsus 1 with seven to eight pseudosegments, mostly distinct.

Variation (male): Tibia 1 in five males (including holotype): 1.35–1.40 (mean 1.37).

Female: In general, similar to male but sternum without pair of anterior humps, chelicerae apparently without stridulatory ridges, and clypeus unmodified. Tibia 1 in nine females: 1.30–1.45 (mean 1.36). Epigynum (Fig. 32A) anterior plate weakly protruding, semi-circular, with almost straight posterior margin; posterior plate large, simple. Internal genitalia (Figs 31I, 32B–D) with indistinct semi-circular receptacle.

Distribution: Known from type locality only, near Nono in Córdoba, Argentina (Fig. 3).

Natural history: The spiders were found among the stones of a loosely built stone wall in the plain sun in a relatively arid environment (Fig. 45F). In small containers in laboratory conditions, the spiders built flimsy webs. Like other pholcids, they hang upside down in these webs. Copulation attempts were not successful. In one of them (25 Feb. 2021), the female attacked the male and started to wrap him.

NERUDIA ATACAMA HUBER, 2000

(FIGS 33, 34)

Nerudia atacama Huber, 2000: 87, figs 333–337.
Nerudia atacama – Torres *et al.*, 2015: 5, fig. 4C, D (see *N. poma*; misidentification).

Diagnosis: Distinguished from known congeners by shape of procursus (Fig. 33A–C; distal half bent towards dorsal; same width over most of its length in lateral view), by bulbal processes (Fig. 33D–F; embolus slender tubular), by armature of male chelicerae (Fig. 33G, H; frontal apophyses at half length, pointing downwards, with pointed tip in frontal view, set with regular hairs; similar to *N. flecha*), and by epigynum and female internal genitalia (Fig. 34; epigynal plate with large posterior indentation, similar to *N. flecha*; internal genitalia with large round median 'receptacle').

Type material: CHILE – **Atacama**: • ♂ holotype, re-examined; S of Domeyko, Cuesta Pajonales; 29.151° S, 70.980° W; 1200 m a.s.l.; 5 Oct. 1992; N. I. Platnick, P. Goloboff, K. Catley leg.; AMNH • 3 ♀♀ paratypes, re-examined; same data as holotype; AMNH.

Other material (not re-examined): CHILE – **Atacama**: • 1 ♀; Cuesta Pajonales, S of Domeyko; 29.146° S, 70.997° W; 1080 m a.s.l.; 5 Oct. 1992; N. I. Platnick, P. Goloboff, K. Catley leg.; AMNH.

Description (amendments; see Huber 2000*):* The distinctive male and female structures are shown (Figs 33, 34) in order to facilitate comparison with the newly described congeners.

Distribution: Known only from the type locality in Atacama, Chile (Fig. 3).

NERUDIA FLECHA HUBER **SP. NOV.**

(FIGS 35, 36)

Zoobank registration: urn:lsid:zoobank.org:act:2A718011-CA44-43E3-8053-166B6AF843DF.

Diagnosis: Easily distinguished from known congeners by shape of procursus (Fig. 35A–C; short distal element with hooked tip, without membranous part) and by bulbal processes (Fig. 35D–F; embolus much shorter than ventral apophysis); also by armature of male chelicerae (Fig. 35G, H; frontal apophyses pointing downwards, with slightly widened flat tip, set with regular hairs), and by epigynum and female internal genitalia (Figs 35I, 36; epigynal plate with large posterior indentation; internal genitalia apparently without or with small median 'receptacle').

Type material: CHILE – **Coquimbo**: • ♂ holotype, 1 ♀ paratype; road to Pascua Lama Mine; approximately 29.445° S, 70.502° W, +/– 6 km; 3000–3280 m a.s.l.; 3 Feb. 2014; A. A. Ojanguren-Affilastro, J. Pizarro-Araya, P. Agusto, R. Botero Trujillo and H. Iuri leg.; *MACN Ar 37782*.

Etymology: The species epithet *flecha* (Spanish for 'arrow') is taken from Pablo Neruda's poem 'Poema 1'; noun in apposition.

Description

Male (holotype). Measurements: Total body length 1.50, carapace width 0.62. Distance PME–PME 80 µm; diameter PME 50 µm; distance PME–ALE 20 µm; distance AME–AME 20 µm; diameter AME 35 µm. Leg 1: 4.57 (1.30 + 0.20 + 1.30 + 1.30 + 0.47), tibia 2: 1.07, tibia 3: 0.90, tibia 4: 1.27; tibia 1 L/d: 19.

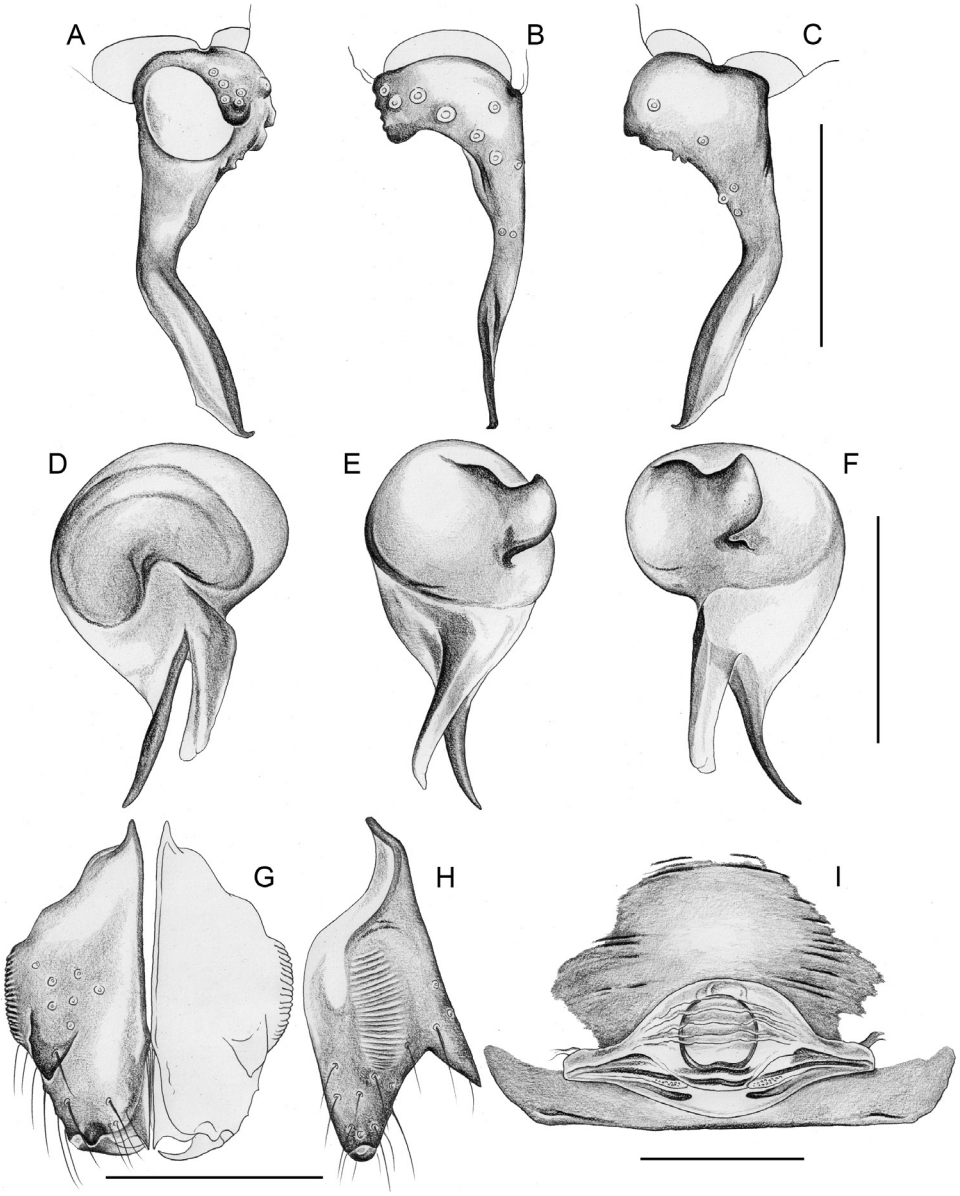

Figure 33. *Nerudia atacama*; holotype male and paratype female from Chile, Atacama, S of Domeyko (AMNH). A–C, left male palpal tarsus and procursus, prolateral, dorsal and retrolateral views. D–F, left male genital bulb, prolateral, dorsal, and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Scale lines: 0.2 mm.

Colour (in ethanol): Prosoma and legs mostly pale ochre-yellow; carapace with light brown Y-mark behind ocular area; legs without dark rings; abdomen monochromous pale-grey.

Body: Habitus similar to *N. poma* (cf. Fig. 1B). Ocular area barely raised. Carapace with indistinct thoracic groove. Clypeus unmodified (only rim slightly more sclerotized than in female). Sternum wider than long (0.44/0.40), with pair of small anterior processes near coxae 1. Abdomen globular.

Chelicerae: As in Figure 35G, H; short frontal apophyses set with regular hairs, tips slightly flattened; stridulatory files on low lateral protrusions.

Palps: In general, similar to *N. colina* (cf. Fig. 4) but femur absolutely and relatively shorter (length/width 1.76) and tibia slightly less strongly enlarged (length/width 1.13); procursus simple, in lateral view slightly bent towards dorsal, distal part short, with distinctive hooked tip, without membranous element (Fig. 35A–C); genital bulb with weakly curved ventral apophysis

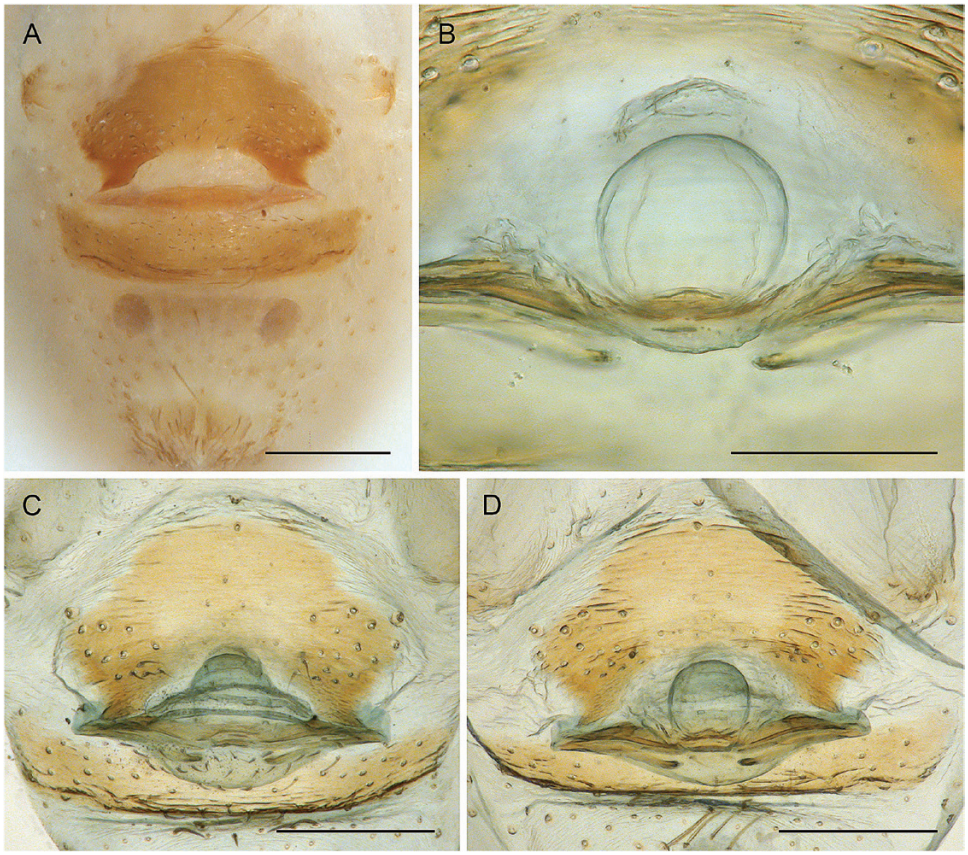

Figure 34. *Nerudia atacama*; paratype female from Chile, Atacama, S of Domeyko (AMNH). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia. C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

distally semi-transparent, embolus much shorter than ventral apophysis (Fig. 35D–F).

Legs: Without spines and curved hairs; with vertical hairs in higher than usual density on tibia 1 only, in two dorsal rows (prolateral and retrolateral); retrolateral trichobothrium of tibia 1 at 64%; prolateral trichobothrium absent on tibia 1; tarsus 1 with ~seven pseudosegments, only distally distinct.

Female: In general, similar to male but sternum without humps and tibia 1 with usual low number of short vertical hairs. Tibia 1: 1.22; carapace width: 0.68. Epigynum (Fig. 36A) anterior plate semi-circular to trapezoidal, with large posterior indentation; posterior plate short but wide. Internal genitalia (Figs 35I, 36B–D) simple, apparently without or with small median 'receptacle'.

Distribution: Known only from type locality in Coquimbo, Chile (Fig. 3).

PUTATIVE FURTHER SPECIES

NERUDIA SP. 'ARG23A'

Three species were collected in San Juan, ~35 km W Las Flores: *N. rocio*, *N. ola* and nine females representing a third species. These females clearly differ from the two named species at this locality by the shape of the epigynum (Fig. 37A; resembles *N. flecha*); from *N. rocio* also distinguished by much shorter legs (tibia 1 length in seven females: 1.10–1.30; mean 1.21).

Material examined. ARGENTINA – **San Juan**: • 3 ♀♀; ~35 km W Las Flores; 30.3967° S, 69.5576° W; 2910 m a.s.l.; 6 Mar. 2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Ar 23911 • 5 ♀♀, in pure ethanol (two prosomata used for molecular study); same data as preceding; ZFMK Arg147; 1 ♀, with egg-sac, in pure ethanol; same data as preceding; LABRE-Ar 562.

NERUDIA SP. 'ARG163'

Two species were collected in La Rioja, SE Aimogasta: *N. ola*, and a single female specimen representing a

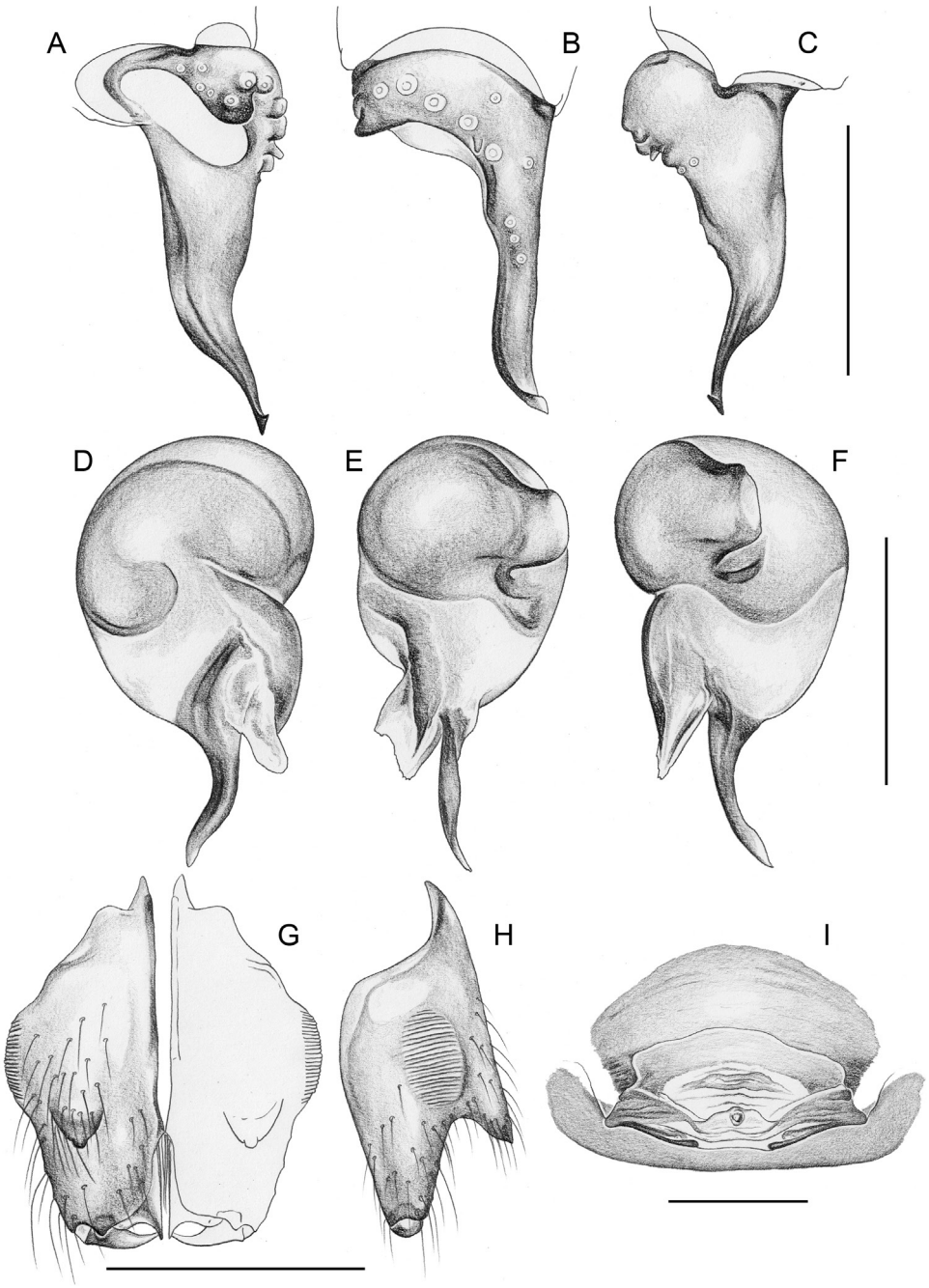

Figure 35. *Nerudia flecha* sp. nov.; holotype male and paratype female from Chile, Coquimbo, road to Pascua Lama Mine (MACN Ar 37782). A–C, left male palpal tarsus and procursus, prolateral, dorsal and retrolateral views. D–F, left male genital bulb, prolateral, dorsal and retrolateral views. G, H, male chelicerae, frontal and lateral views. I, cleared female genitalia, dorsal view. Scale lines: 0.2 mm.

different species. This female resembles *N. rocio* in having indistinct radial marks on the carapace and in having unusually long legs (tibia 1: 2.1). However, the epigynum is different (Fig. 37B) and resembles that of *N. hoguera* (and *N. flecha*). The molecular data also suggest that this specimen is closer to *N. hoguera* than to any other sequenced species (Fig. 2; Supporting Information, Fig. S4).

Material examined. ARGENTINA – **La Rioja**: • 1 ♀, in pure ethanol; SE Aimogasta, 'site 2'; 28.9015° S, 66.6538° W; 755 m a.s.l.; under rocks; 10 Mar.

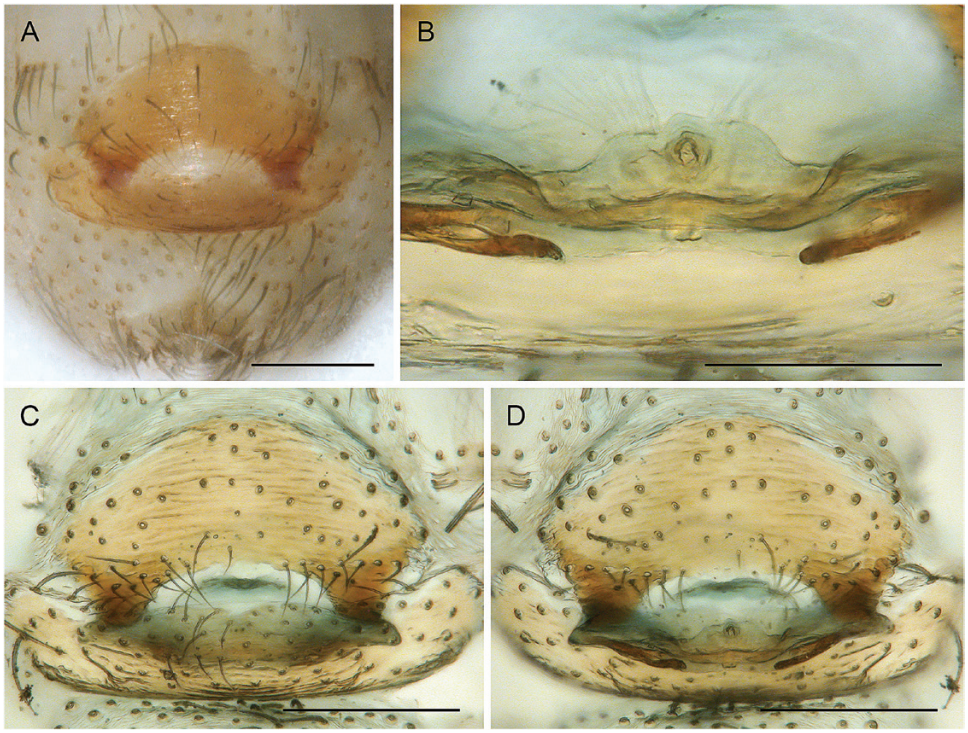

Figure 36. *Nerudia flecha* sp. nov.; paratype female from Chile, Coquimbo, road to Pascua Lama Mine (MACN Ar 37782). A, abdomen and epigynum, ventral view. B, median structures in internal genitalia. C, D, cleared genitalia, ventral and dorsal views. Scale lines: 0.2 mm (A, C, D), 0.1 mm (B).

2019; B. A. Huber and M. A. Izquierdo leg.; ZFMK Arg163.

KARYOLOGY

The male karyotype of *Nerudia ola* is composed of 24 chromosomes. It is predominated by biarmed chromosomes (Fig. 38A). The prophase of the first meiotic division includes the diffuse stage. During this period, the bivalents almost decondense. In contrast to this, sex chromosomes form a highly condensed, cross-shaped element (Fig. 38B). A diffuse stage is also found in *N. poma* and *Gertschiola macrostyla*. The metaphase of the first meiotic division (metaphase I) consists of ten bivalents and a tetravalent formed by three X chromosomes and a Y chromosome (Fig. 38C, D). Within the tetravalent, each of the X chromosomes is associated by one end with the Y chromosome (Fig. 38C). The average size of the Y chromosome (8.3 µm, $N = 3$, metaphases I) is similar to the size of the X chromosomes. There are two types of metaphases of the second meiotic division (metaphase II), one with 11 chromosomes, including the Y chromosome, and the other with 13 chromosomes, including the X chromosomes (Fig. 38E, F). It was impossible to distinguish the sex chromosomes from the other chromosomes on the basis of their morphology or behaviour during metaphase II (Fig. 38E, F). One (Fig. 39B, C) or two chromosome pairs (Fig. 39A) contain a NOR. One sex chromosome also included a NOR (Fig. 39C), at the end involved in chromosome pairing (Fig. 39B).

The male karyotype of *Nerudia poma* consists of 26 chromosomes (Fig. 40A). Metaphases I contain one bivalent more than in *N. ola* (Fig. 40B, C). The structure of the sex chromosome tetravalent is the same as in *N. ola* (Fig. 40B, C) except for the smaller size of the Y chromosome (5.1 µm, $N = 2$, metaphases I). There are two types of metaphases II, one with 12 chromosomes, including the tiny Y chromosome, and another with 14 chromosomes, including the three X chromosomes (Fig. 40D–F). It was impossible to distinguish the X chromosomes from the other chromosomes at metaphase II (Fig. 40D, F). Metaphase II with X chromosomes is formed exclusively by biarmed chromosomes (Fig. 41), which implies that all chromosome pairs and the X chromosomes are biarmed. The Y chromosome is the smallest element of metaphase II (Fig. 40E). Its morphology is unknown.

The male karyotype of *Gertschiola macrostyla* is predominated by biarmed chromosomes (Fig. 42D). Metaphases I contain 12 bivalents and a sex chromosome tetravalent $X_1X_2X_3Y$. The X chromosomes are

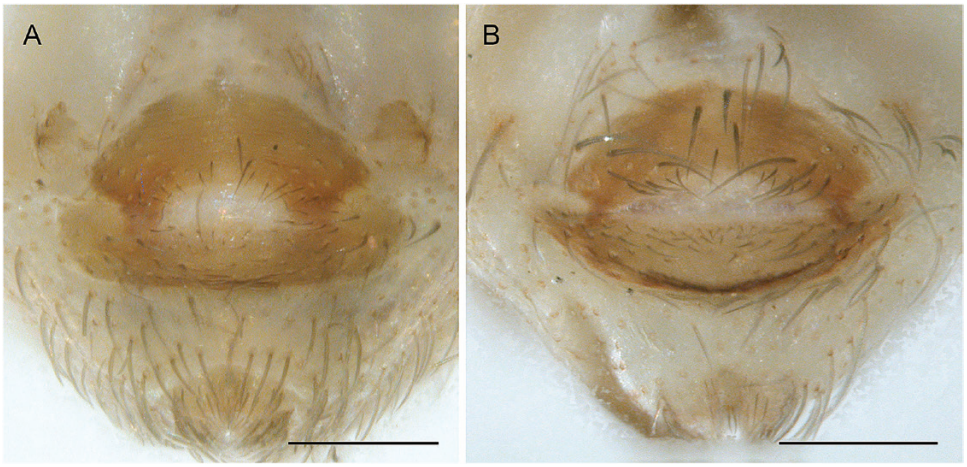

Figure 37. Epigyna of two formally undescribed putative species, ventral views. A, *Nerudia* 'Arg23a' from Argentina, San Juan, 35 km W Las Flores (ZFMK Ar 23911). B, *N.* 'Arg163' from Argentina, La Rioja, SE Aimogasta, 'site 2' (ZFMK Arg163). Scale lines: 0.2 mm.

associated by the ends of both of their arms with the tiny Y chromosome, which is much smaller than in *Nerudia* (Fig. 42A–C). The average size of the Y chromosome is only 1.6 µm ($N = 2$, metaphases I). One chromosome pair contains a NOR. Moreover, one X chromosome has a terminal NOR (Fig. 39D).

Sampling bias and biogeographic analyses

The areas with high densities of records of arthropods and arachnids are poorly correlated (r = 0.391, $P < 0.000$). In the wider area (~500 km) around the known geographic distribution of *Nerudia*, six localities stand out for their high density of records of arthropods, near the cities of Santiago, La Serena and Copiapó in Chile, and Córdoba, Cafayate and Salta in Argentina (Supporting Information, Fig. S5). For arachnids, only the cities of Santiago, Cafayate and Córdoba present a similar pattern, while an extra area with high density of records of Arachnida is observed near Tilcara, in northern Argentina (Fig. 43A). Meanwhile, the records of *Nerudia* are mostly located in poorly sampled regions (Fig. 43A), and they are not explained by the number of records of arthropods (deviance = 1.513, $P = 0.2187$) or by the interaction between the numbers of records of arthropods and arachnids (deviance = 2.577, $P = 0.1084$). However, records of *Nerudia* are significantly explained by the number of records of arachnids (deviance = 5.447, $P = 0.019$; Supporting Information, Fig. S6).

The species distribution modelling was based on seven principal components (PC) of the 21 predictor bioclimatic variables, which gathered 95.3% of the cumulative proportion of variance (for details, see Supporting Information, Tables S1, S2). The first PC contributed with 43% to the *Nerudia* distribution modelling (Fig. 43B; Supporting Information, Table S3), while, the second PC contributed with 33.6% (see Table S3). Most of the areas with higher relative occurrence rates for *Nerudia* (Fig. 43B) are in the northern part of the Monte biogeographic province (*sensu* Morrone, 2017) (where most *Nerudia* records are known) and in the Atacama province in Chile and the Puna province in Bolivia (where no *Nerudia* or any other Ninetinae have ever been recorded).

The second principal component analysis carried out based on the extracted values for the 21 predictor bioclimatic variables for each of the Ninetinae records (Supporting Information, Table S6), resulted in eight principal components that gather 96.0% of the cumulative proportion of variance (Supporting Information, Table S4). The first PC represented 47.7% of the proportion of variance, being positively correlated with temperature seasonality and temperature annual range, but negatively correlated with annual mean temperature, minimum temperature of coldest month and mean temperature of coldest quarter (Supporting Information, Table S5; Fig. S7). The second PC represented 19.4% of the proportion of variance, being positively correlated with the annual precipitation and the precipitation of the driest month, of the driest quarter and of the coldest quarter, but negatively correlated with precipitation seasonality (Supporting Information, Table S5). The ordination of the *Nerudia* records in the multivariate space (Fig. 44; Supporting Information, Fig. S7) indicates that this taxon is likely limited by extreme cold conditions and humid environments. Representatives of *Nerudia* occupy regions

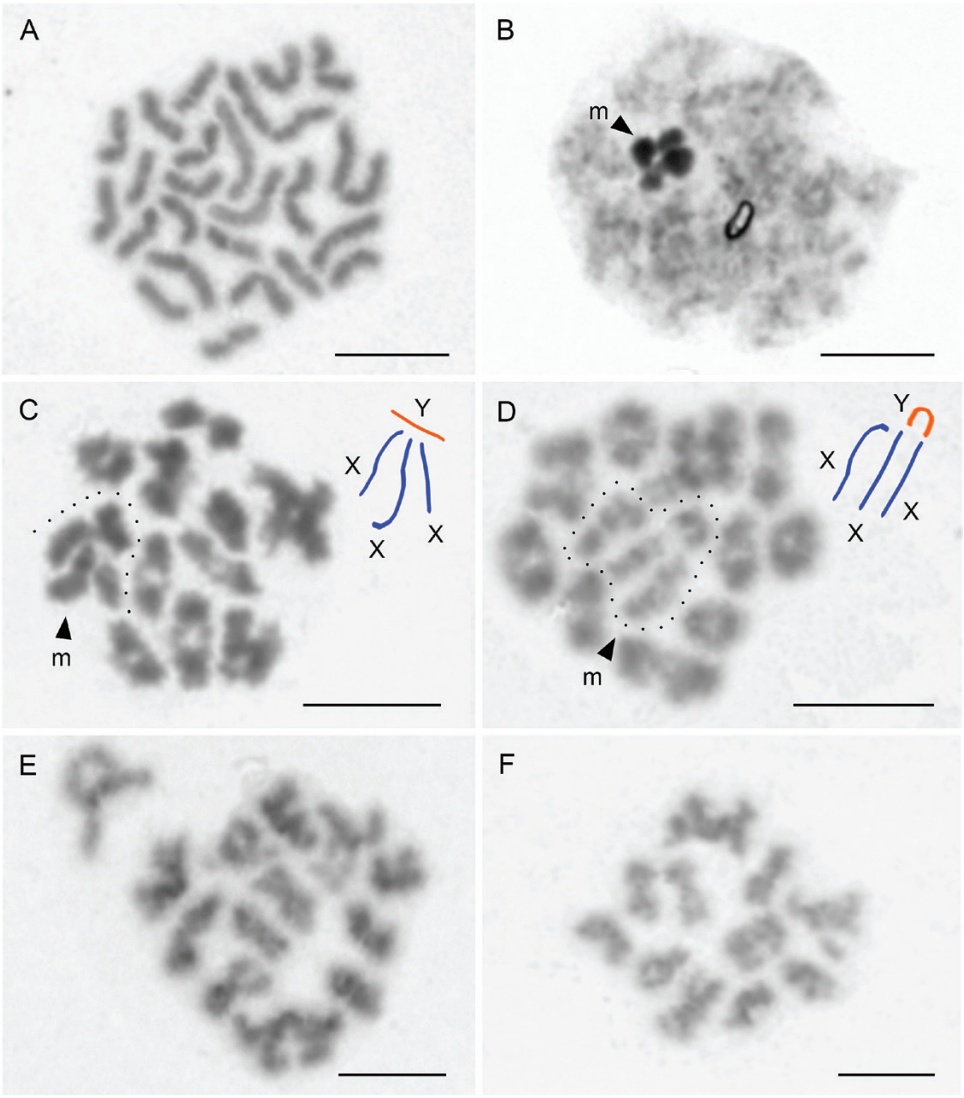

Figure 38. *Nerudia ola* sp. nov.; male chromosome plates. Panels C, D contain a scheme of the sex chromosome multivalent (blue – X chromosomes, orange – Y chromosome). A, mitotic metaphase, $2n = 24$. Note the predomination of biarmed chromosomes. B, diffuse stage. Note the cross-shaped multivalent consisting of four sex chromosomes. The multivalent exhibits positive heteropycnosis. C, D, metaphases I consisting of ten bivalents and a sex chromosome multivalent comprising three X chromosomes and a Y chromosome. Multivalent delimited by dotted line. One X chromosome is not included in the multivalent at D. E, early metaphase II containing the X chromosomes ($n = 13$). It is impossible to distinguish the X chromosomes from other chromosomes. F, early metaphase II containing the Y chromosome ($n = 11$). It is impossible to distinguish the Y chromosome from other chromosomes. Abbreviations: m, sex chromosome multivalent; X, X chromosome; Y, Y chromosome. Scale lines: 10 µm.

with lower tree density and lower tree canopy height (Supporting Information, Fig. S7), suggesting an association with open environments. However, such biotic and climatic conditions are not significantly different from those observed for most other Ninetinae; the *Nerudia* records are within the confidence interval for other taxa (Fig. 44). The environmental niche is phylogenetically conserved in the group, thus evolving as expected owing to Brownian motion (Pagel's $\lambda = 0.95$, P-value = 0.000, for the first PC axis; full results in Supporting Information, Fig. S8, Table S9).

DISCUSSION

KARYOLOGY

Only two representatives of Ninetinae have been karyotyped before, *Kambiwa neotropica* (Kraus, 1957) and *Pholcophora americana* Banks, 1896 (Ávila Herrera

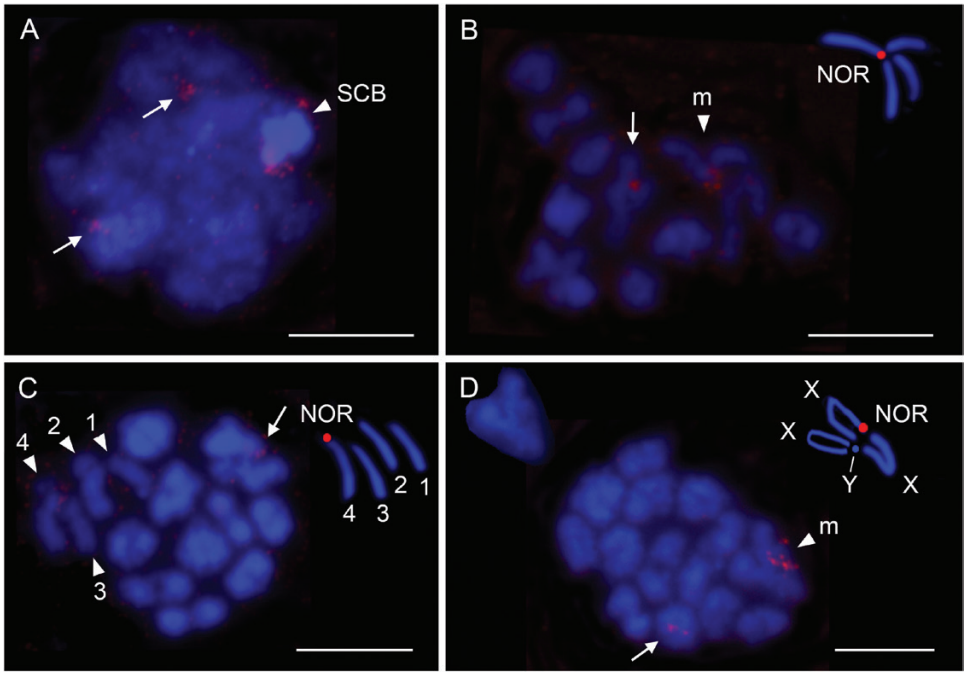

Figure 39. *Nerudia ola* sp. nov. (A–C) and *Gertschiola macrostyla* (D); male meiosis, detection of nucleolus organizer regions (NORs; red signals). Chromosomes counterstained with DAPI (blue). Panels B–D contain schemes of the sex chromosome multivalents. A, diffuse stage. Sex chromosome body and two decondensed bivalents exhibit a signal. B, metaphase I, one bivalent includes a NOR. Another NOR is at the end of a sex chromosome involved in pairing. C, metaphase I, sex chromosome multivalent collapsed. One bivalent and one end of a sex chromosome bear a NOR. D, metaphase I. One bivalent and one end of an X chromosome contains a NOR. Note the sex chromosome multivalent in the upper left corner of the panel. Symbols: arrow, NOR-bearing bivalent; arrowhead, sex chromosome body (A), sex chromosome multivalent (B, D) or particular sex chromosomes (C, numbered). Abbreviations: m, sex chromosome multivalent; SCB, sex chromosome body; X, X chromosome; Y, Y chromosome. Scale lines: 10 µm.

et al., 2021). In this study, we added three further species and two genera, namely *Nerudia poma*, *N. ola* and *Gertschiola macrostyla*. Closely related pholcids often differ in the morphology of some chromosomes (Ávila Herrera *et al.*, 2021). Unfortunately, we were only able to determine the morphology of the chromosomes for *Nerudia poma* (except for the Y chromosome). As in the vast majority of other haplogyne araneomorphs with monocentric chromosomes (Král *et al.*, 2006), karyotypes of ninetines are predominated by biarmed chromosomes (Ávila Herrera *et al.*, 2021; this study). The male prophase of the first meiotic division includes the so-called diffuse stage (Ávila Herrera *et al.*, 2021; this study), which is probably a synapomorphy of haplogyne spiders, i.e. the clade formed by Synspermiata and two cribellate families, Filistatidae and Hypochilidae (Ávila Herrera *et al.*, 2021).

Among Ninetinae, the karyotype of *P. americana* ($2n♂ = 29, X_1X_2Y$) is supposedly close to the ancestral karyotype of pholcids ($2n♂ = 33, X_1X_2Y$; Ávila Herrera *et al.*, 2021) in terms of diploid number and sex chromosome system. The X_1X_2Y system of *P. americana* includes two large metacentric X chromosomes and a metacentric Y microchromosome, which pair without chiasmata by the ends of their arms during male meiosis (Ávila Herrera *et al.*, 2021). This system has been found in seven haplogyne families (Silva, 1988; Silva *et al.*, 2002; Král *et al.*; 2006, 2019; Ávila Herrera *et al.*, 2016; Paula-Neto *et al.*, 2017; Araujo *et al.*, 2020), including some pholcid clades (Král *et al.*, 2006; Ávila Herrera *et al.*, 2021). It is probably the ancestral sex chromosome determination of araneomorph spiders, including haplogynes (Paula-Neto *et al.*, 2017; Ávila Herrera *et al.*, 2021).

In contrast to the North American *P. americana*, the karyotypes of the South American *K. neotropica* (Ávila Herrera *et al.*, 2021), *G. macrostyla* and *Nerudia* spp. (this study) contain fewer chromosome pairs. The latter three genera belong to the same clade, separate from *Pholcophora* (Huber *et al.*, 2018). Our data thus suggest that the ancestral karyotype of this South American clade was characterized by a reduction in the number of chromosome pairs. According to our data, the ancestral karyotype of the clade including

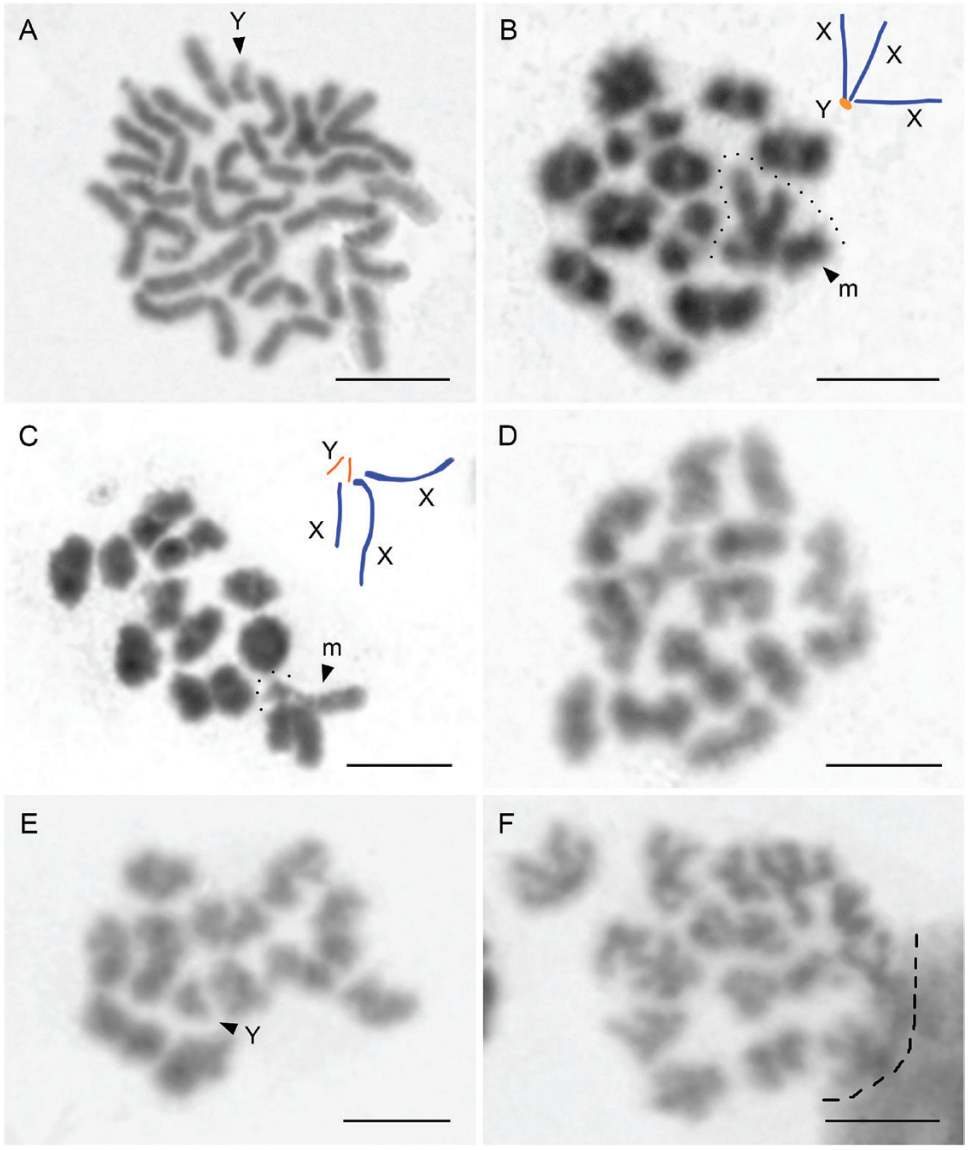

Figure 40. *Nerudia poma* sp. nov., male chromosome plates. Panels B, C contain a scheme of the sex chromosome multivalent (blue – X chromosomes, orange – Y chromosome). A, mitotic metaphase, $2n = 26$. Note the predomination of biarmed chromosomes. The Y chromosome is the smallest element of the complement. B, C, metaphases I consisting of 11 bivalents and a sex chromosome multivalent comprising three X chromosomes and a Y chromosome. Multivalent delimited by dotted line. D, metaphase II containing X chromosomes ($n = 14$). It is impossible to distinguish the X chromosomes from other chromosomes. E, early metaphase II containing the Y chromosome ($n = 12$). The Y chromosome is the smallest chromosome of the plate. F, late metaphase II containing the X chromosomes ($n = 14$). Note the predomination of biarmed chromosomes. This plate was used to construct the haploid karyotype of the species (Fig. 41). Abbreviations: m, sex chromosome multivalent; X, X chromosome; Y, Y chromosome. Scale lines: 10 µm.

Gertschiola, *Kambiwa* Huber, 2000 and *Nerudia* contained 12 chromosome pairs. This number is retained in *Gertschiola* (this study) and *Kambiwa* (Ávila Herrera *et al.*, 2021). A further reduction occurred in *Nerudia*, where this process can even be observed within the genus (this study). The reduction of chromosome numbers is a frequent phenomenon in the evolution of various spider groups (Suzuki, 1954; Kořínková & Král, 2013), including pholcids (Ávila Herrera *et al.*, 2021).

The karyotypes of *Kambiwa*, *Gertschiola* and *Nerudia* further differ from that of *Pholcophora* by the more complex sex chromosome systems (Ávila Herrera *et al.*, 2021; this study). *Gertschiola* and

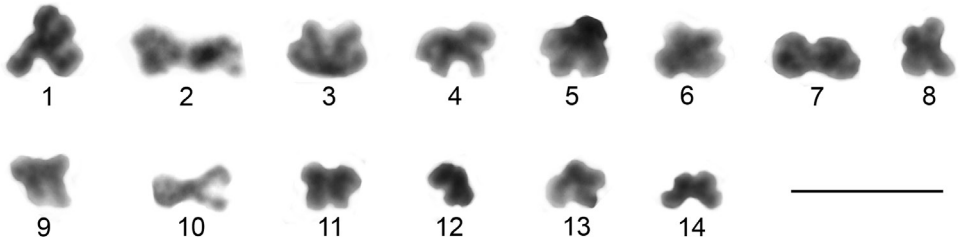

Figure 41. *Nerudia poma* sp. nov., haploid karyotype (n = 14) based on metaphase II containing X chromosomes. It consists of nine metacentric (nos 1–8, 10) and five submetacentric chromosomes (nos 9, 11–14). It is impossible to distinguish X chromosomes on the basis of their morphology or behaviour. Scale line: 10 µm.

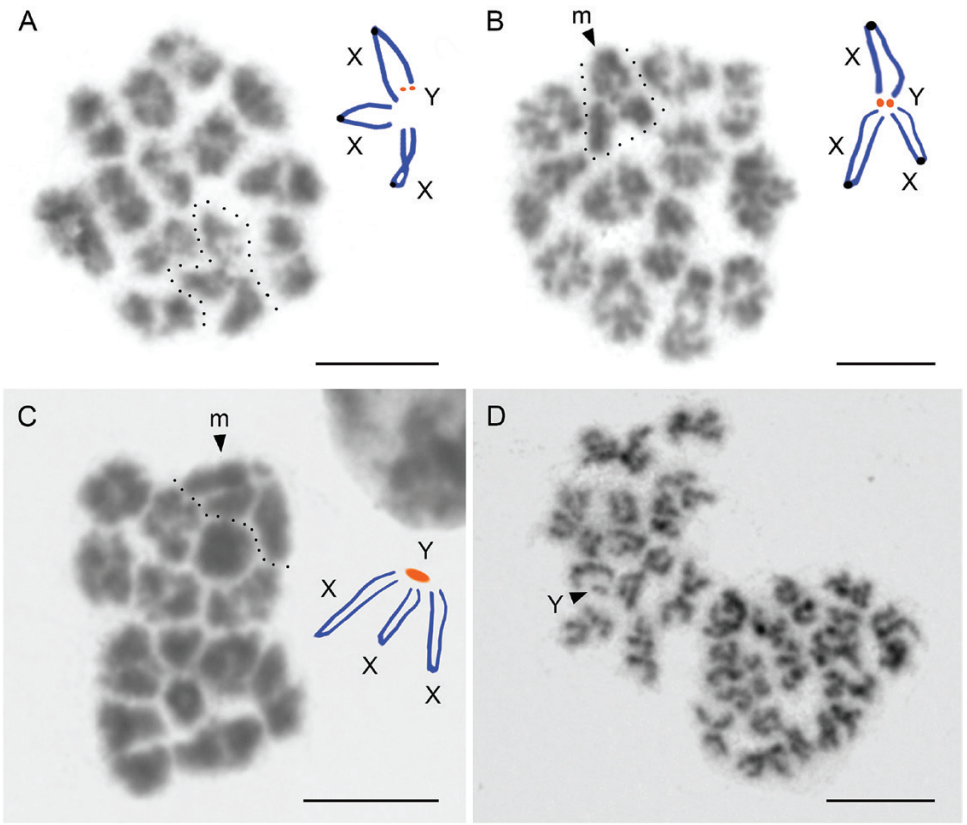

Figure 42. *Gertschiola macrostyla* sp. nov.; male chromosome plates. Panels A–C contain a scheme of the sex chromosome multivalent (blue – X chromosomes, orange – Y chromosome, black knob – supposed centromere). A–C, metaphases I consisting of 12 bivalents and a sex chromosome multivalent comprising three X chromosomes and a Y chromosome. Multivalent delimited by dotted line. D, plate composed of two sister metaphases II. Note the tiny Y chromosome. Abbreviations: m, sex chromosome multivalent; X, X chromosome; Y, Y chromosome. Scale lines: 10 µm.

Nerudia share the $X_1X_2X_3Y$ system (this study), which has not previously been reported for pholcids. We suppose that it evolved from the X_1X_2Y system, as has been hypothesized for many other sex chromosome systems previously described in pholcids (Ávila Herrera *et al.*, 2021). Chromosomes of the $X_1X_2X_3Y$ system retained achiasmatic pairing during male meiosis (this study). Furthermore, the mode of pairing of the X chromosomes in *Gertschiola* (see below) suggests that these elements retained their biarmed morphology. The third X chromosome of the $X_1X_2X_3Y$ system could arise by a nondisjunction of one of the X chromosomes, which is supposed to be the mechanism of formation of multiple X chromosomes in entelegyne araneomorphs (Postiglioni & Brum-Zorrilla, 1981; Datta & Chatterjee, 1988; Král, 2007).

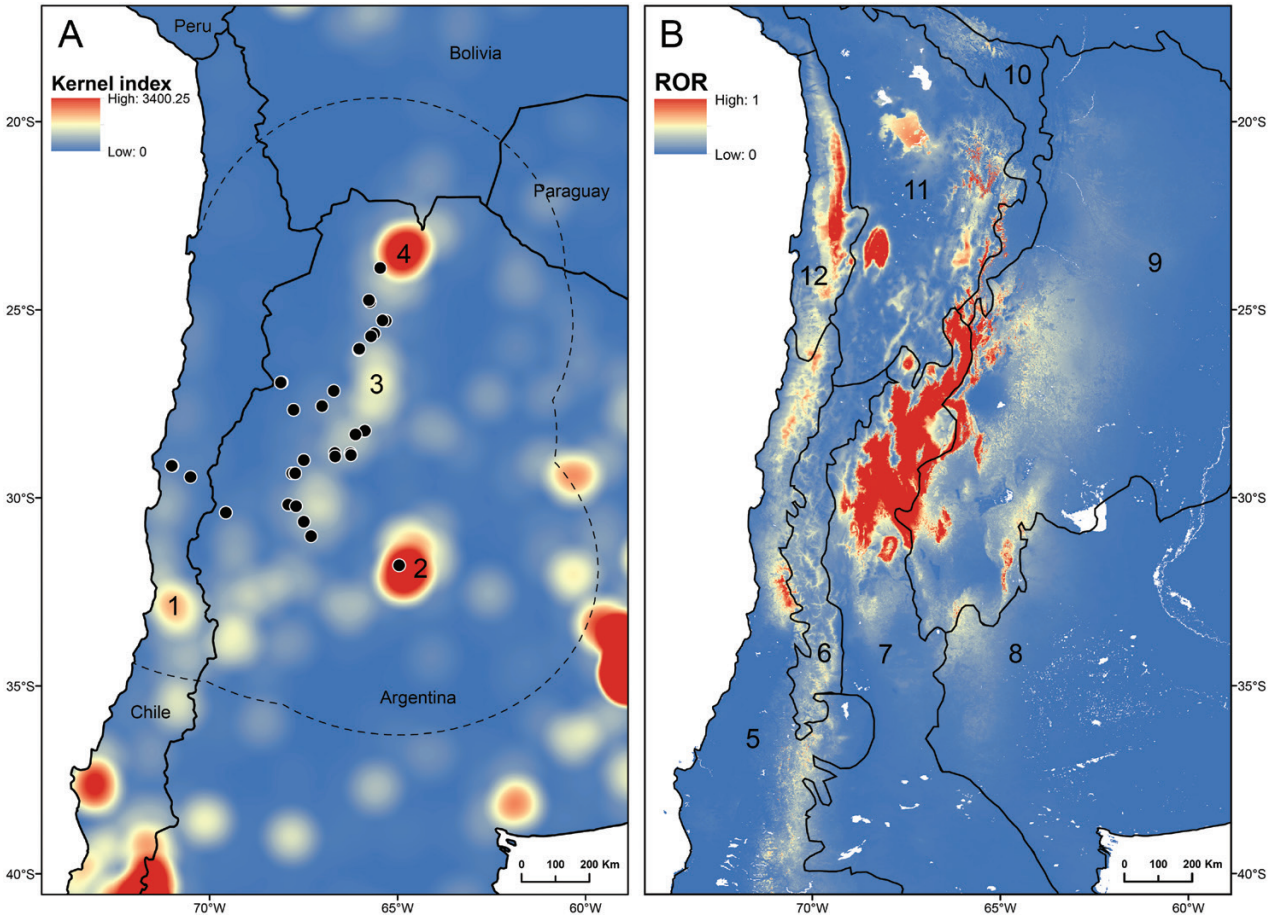

Figure 43. A, density of records of arachnids surrounding the geographic distribution of *Nerudia* representatives, with numbered high density regions (reddish areas: 1. Santiago; 2. Córdoba; 3. Cafayate; 4. Tilcara). Dotted line represents 500 km buffer around *Nerudia* records (black dots). Note that *Nerudia* records are concentrated in poorly sampled regions (bluish areas). B, relative occurrence rate (ROR) of *Nerudia* species, with numbered biogeographic regions. Polygons represent the biogeographical regionalisation (*sensu* Morrone, 2017) in the Andean (5) and Neotropical (6–12) regions. Neotropical provinces: (6) Prepuna; (7) Monte; (8) Pampean; (9) Chaco; (10) Yungas; (11) Puna; and (12) Atacama. The reddish areas denote regions where *Nerudia* is expected to occur; in some of these, no Ninetinae have ever been recorded: the Atacama province in Chile and the Puna province in Bolivia.

Alternatively, it could originate by a fission of one metacentric X chromosome of the X_1X_2Y system followed by a pericentric inversion of arising elements (to restore their biarmed morphology). The same complex rearrangement probably transformed the X_1X_20 system into $X_1X_2X_30$ in the pholcid *Smeringopus pallidus* (Blackwall, 1858) (Ávila Herrera *et al.*, 2021). Fissions have also been involved in sex chromosome evolution of some mygalomorph spiders (Král *et al.*, 2013). In *Gertschiola*, the X chromosomes still pair in meiosis by both ends and the Y chromosome retains its tiny size even after the formation of the $X_1X_2X_3Y$ system. The details of chromosome pairing in *Nerudia* remain unresolved. Each X chromosome can possibly associate by one of its ends with the Y chromosome as stated in the Results section. In another possible arrangement, each of the X chromosomes would have its arms closely aligned together and thus make the impression of pairing with the Y chromosome by one end. This is common in species with the X_1X_2Y system (Ávila Herrera *et al.*, 2021). *Nerudia* also exhibits an increase in the size of the Y chromosome. This usually results from incorporation of autosome material (e.g. Schartl *et al.*, 2016) or from expansion of heterochromatin (e.g. Kejnovský *et al.*, 2009). In *Nerudia ola*, the Y chromosome reaches the same size as the X chromosomes. The considerable extension of the Y chromosome in *Nerudia* has been

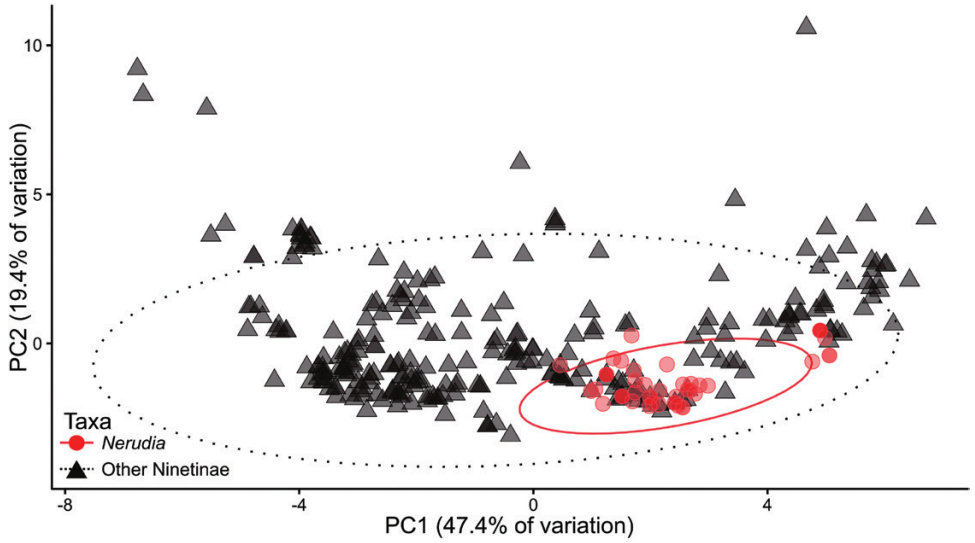

Figure 44. Principal component analysis of the environmental conditions for *Nerudia* representatives (red circles and ellipse) and other Ninetinae (black triangles and dotted line ellipse). The ellipses encompass the values within a 95% confidence interval of a multivariate t-distribution. Darker symbols result from superimposition. Note that the environmental conditions for *Nerudia* representatives are within the confidence interval for other ninetines, suggesting that the environmental niche of *Nerudia* is not significantly different from other Ninetinae taxa.

accompanied by a decrease in the number of chromosome pairs, which indicates integration of chromosome fragment(s) into the Y chromosome. Accretion of the Y chromosome has also been observed in some haplogyne spiders with the X_1X_2Y system, namely in some other pholcid lineages (Ávila Herrera *et al.*, 2021) and a representative of the family Pacullidae (Král *et al.*, 2019).

Kambiwa neotropica has an $X_1X_2X_3X_4Y$ system (Ávila Herrera *et al.*, 2021). This could originate from the X_1X_2Y system by inclusion of one chromosome pair (Ávila Herrera *et al.*, 2021). In this scenario, the $X_1X_2X_3Y$ system of *Gertschiola* and *Nerudia* arose from the $X_1X_2X_3X_4Y$ system by a centric fusion of the mono-armed chromosomes X_3 and X_4. Another plausible scenario is that the $X_1X_2X_3X_4Y$ system evolved from the $X_1X_2X_3Y$ system by a non-disjunction or fission of one biarmed X chromosome. This second scenario is favoured by the behaviour of chromosomes of the $X_1X_2X_3X_4Y$ system during male meiosis. They exhibit a similar pattern of heteropycnosis during this period. If some chromosomes of the $X_1X_2X_3X_4Y$ system had arisen from a chromosome pair, they would have retained their original meiotic behaviour and would therefore lack heteropycnosis. Therefore, the $X_1X_2X_3Y$ system is probably an ancestral character of the South American clade containing the genera *Gertschiola*, *Kambiwa* and *Nerudia*.

A further character supporting the monophyly of this clade with an $X_1X_2X_3X_4Y$ or $X_1X_2X_3Y$ system is an X chromosome-linked NOR. While the number of NORs varies considerably in pholcids (from one to nine), the position of NORs in these spiders is conservative. Almost all analysed species have terminal NORs. This pattern probably reflects the spreading of rDNA by ectopic recombinations (Ávila Herrera *et al.*, 2021). It has been suggested that ancestral ninetines had two NOR-bearing chromosome pairs (Ávila Herrera *et al.*, 2021). This pattern is preserved in *Nerudia ola*. In *G. macrostyla*, the number of NOR bearing pairs has been reduced to one. As in other haplogynes (Král *et al.*, 2006), NORs in pholcids have often spread to sex chromosomes (Ávila Herrera *et al.*, 2021). Sex chromosome-linked NORs have originated at least five times in Pholcidae (Ávila Herrera *et al.*, 2021). One of these events concerns Ninetinae. While the sex chromosomes of *Pholcophora* (X_1X_2Y) do not include NORs (Avila Herrera *et al.*, 2021), ninetines with more than two X chromosomes exhibit a sex chromosome-linked NOR. In *Gertschiola* and *Kambiwa*, this NOR is placed on an X chromosome (Ávila Herrera *et al.*, 2021; this study). For *Nerudia* we failed to determine unequivocally if a NOR is placed on an X chromosome. Its placement on the X chromosome is supported by the fact that sex chromosome-linked NORs of other studied haplogynes are located on X chromosome(s) (Král *et al.*, 2006; Oliveira *et al.*, 2007) except for the Y-linked NOR of the Pholcinae genus *Nipisa* Huber, 2018 (Ávila Herrera *et al.*, 2021). In contrast to *Kambiwa* (Ávila Herrera *et al.*, 2021), ninetines with the $X_1X_2X_3Y$ system (*Gertschiola* and *Nerudia*) have a sex chromosome-linked NOR at

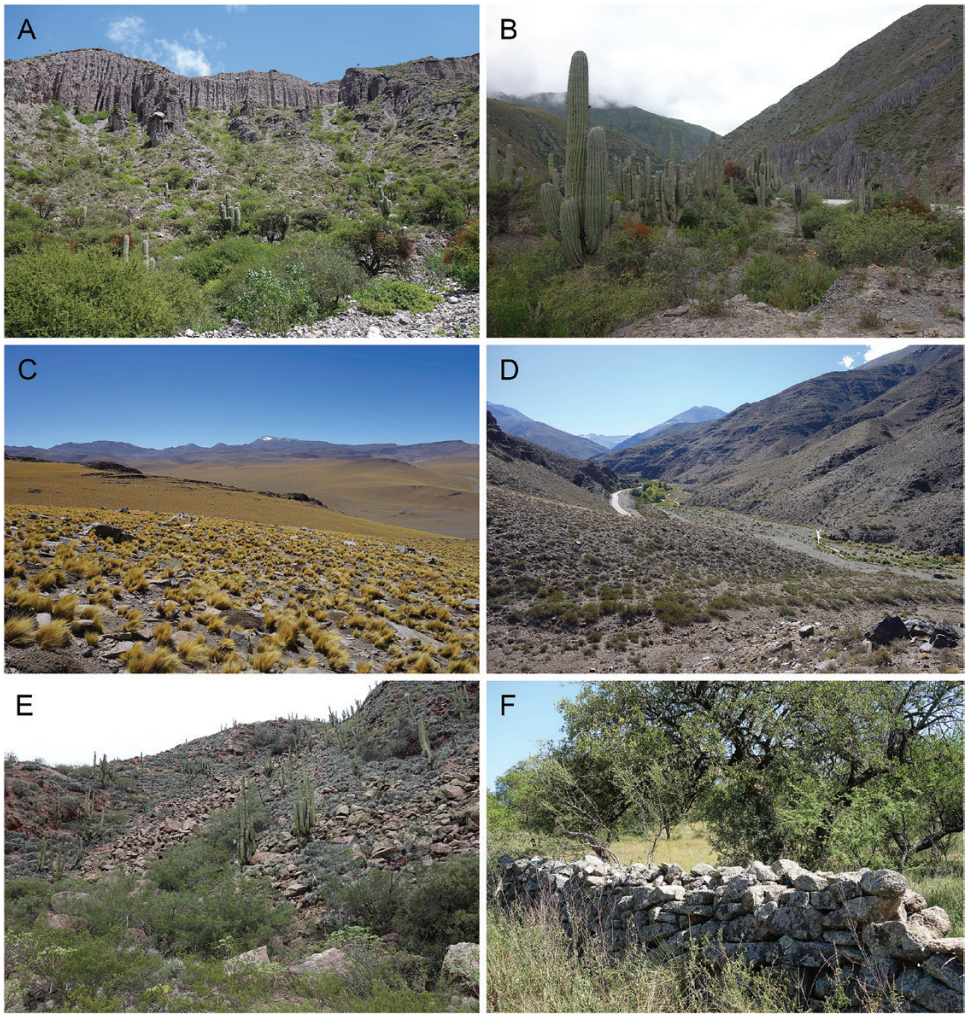

Figure 45. Sample of typical *Nerudia* habitats in Argentina. A, Jujuy, between San Salvador and Purmamarca (type locality of *N. colina* sp. nov.). B, Salta, NW of Campo Quijano (type locality of *N. poma* sp. nov.). C, Catamarca, near Paso de San Francisco (type locality of *N. centaura* sp. nov.). D, San Juan, W of Las Flores (type locality of *N. rocio* sp. nov.). E, La Rioja, SE of Aimogasta (*N. ola* sp. nov.). F, Córdoba, near Nono (type locality of *N. nono* sp. nov.).

the end of the sex chromosome, which is involved in pairing (this study).

Sampling bias and biogeographic analyses

Our knowledge about the geographic distribution of the genus *Nerudia* is greatly improved in the present study. Originally the genus was known from a single record in Chile (Huber, 2000) in the Andean region *sensu* Morrone (2014, 2017). Torres *et al.* (2015) added a second record in the Western Chacoan district in the Chaco province in the Neotropical region. Our new records decrease the Wallacean shortfall in this genus (i.e. the uncertainty regarding its geographic distribution; Hortal *et al.*, 2015) by filling gaps in the Chaco, Cuyan High Andean and Monte provinces (*sensu* Morrone, 2017). However, the records in the Chaco province are mostly located at high-elevation areas that have lower precipitation levels compared to the Eastern district (Morrone, 2017), where no *Nerudia* has been recorded so far. Most of the records of *Nerudia* in the Chaco province are located close to the Monte province, supporting the affinities of these two provinces (Morrone, 2017). The distribution of *Nerudia* within the Chaco province is similar to that observed for Sicariinae spiders, which are also absent in mesic Chaco localities (Magalhaes *et al.*, 2017).

Our results suggest a strong effect of sampling bias on the Linnean (i.e. the uncertainty regarding the number of described species of a taxon; Hortal *et al.*, 2015) and Wallacean shortfalls in this group of spiders. We tested the null hypothesis that *Nerudia*

specimens were found at highly sampled areas for arthropods in general. This could result from a preference for sites visited by previous collectors. Our results do not support this hypothesis. This could, in theory, be explained by the absence of sampling with pitfalls traps in areas where *Nerudia* occurs. This explanation is poorly supported considering the sampling with pitfall traps at Rosario de Lerma (Salta, Argentina), where *Nerudia poma* occurs but was not collected by pitfall traps (Torres *et al.*, 2015). Besides, the correlation between the presence of *Nerudia* and highly sampled areas for arachnids might be due to a mere spurious correlation between these two variables, as 93% of the available specimens of *Nerudia* were directly collected by us (BAH and MAI) in a sampling expedition carried out in 2019. Most newly collected specimens were found by active searching on the undersides of rocks, directly looking for Ninetinae spiders. This exemplifies the importance of research projects focused on decreasing biodiversity shortfalls of target taxa, and the need of specific search methods adapted to those taxa.

The analysis of sampling bias was based on the use of data from the Global Biodiversity Information Facility – GBIF, which includes over 1 billion records (Hughes *et al.*, 2021). The GBIF data is not appropriate for studies that require detailed species lists (e.g. Qian *et al.*, 2022), and it provides less information regarding range filling, range extent and climatic niche of species than independent compilation efforts (e.g. Beck *et al.*, 2013). This is explained by the uneven country-based policies and institutional support for funding and dataset contributions to the platform (Yesson *et al.*, 2007; Beck *et al.*, 2013; Hughes *et al.*, 2021). However, the present study compared the density of records of arthropods and arachnids mostly inside the Argentinean territory, an associate participant country whose head of delegation is an arachnologist (see https://www.gbif.org/the-gbif-network). Therefore, GBIF data availability biases are unlikely to provide false-positive results regarding the effects of sampling bias in *Nerudia* spiders. On the contrary, the sampling bias (e.g. presence of records close to main cities) observed herein, perfectly corroborates comparative studies using large-scale biodiversity data (e.g. Oliveira *et al.*, 2016; Hughes *et al.*, 2021). An additional analytical bias accepted herein results from the decision on using the records of all *Nerudia* species to perform the species distribution modelling. As stated before, this disregards individual species environmental thresholds. However, this is unlikely to provide significant false-positives (i.e. areas with high suitability for *Nerudia* representatives), owing to the phylogenetically conserved environmental niche, which implies that *Nerudia* species share a similar niche.

Many ground-dwelling Pholcidae species, including representatives of Ninetinae, can be collected using pitfall traps. This was reported, for example, for *Ibotyporanga* Mello-Leitão, 1944 in the semiarid Brazilian Caatinga (Huber & Brescovit, 2003), for *Ninetis* Simon, 1890 in desert scrub forests in Madagascar, savannas in Namibia and dunes in Somalia (Huber & El-Hennawy, 2007) and for *Galapa* Huber, 2000 in salt marshes in the Galápagos Islands (Baert, 2014). This agrees with the observation that many ninetines (e.g. *Galapa* Huber, 2000; *Ibotyporanga* Mello-Leitão, 1944; *Kambiwa* Huber, 2000; *Magana* Huber, 2019; *Ninetis*; *Pinoquio* Carvalho & Huber, 2022; *Tolteca* Huber, 2000) run quickly when disturbed and easily drop to the ground (B. A. Huber & L. S. Carvalho, pers. obs.). By contrast, representatives of *Nerudia* live on the undersides of large rocks and seem reluctant to drop to the ground when the rock is turned. We thus hypothesize that representatives of *Nerudia* show specific behaviours related to escape reaction at disturbance that require focused and active sampling, rather than generalist traps and/or passive sampling methods. This hypothesis is corroborated by the positive correlation between the presence of *Nerudia* and records of arachnids in the region. Laboratory and/or field experiments are required to test if the anecdotal observations of behavioural differences reflect statistically significant patterns or not.

The distribution modelling suggests that *Nerudia* is not expected to occur in the low-elevation areas in the Eastern district of the Chaco biogeographic province, although the sampling effort for arachnids in that region is strikingly low (Fig. 43A). The easternmost record of *Nerudia* is located in the surroundings of Córdoba, one of the best sampled regions within the Western district. A high relative occurrence rate for *Nerudia* is predicted for the Atacama biogeographic province in northern Chile and the Puna biogeographic province in southern Bolivia (Fig. 43B). However, these are poorly sampled regions as far as the arachnid fauna is concerned (Fig. 43A), and the only known records of Ninetinae in Chile are those of *Nerudia atacama* and *N. flecha*. Based on our distribution model, we predict that the genus *Nerudia* ranges much farther north in Chile than presently known and into southern Bolivia. On the other hand, a contrasting pattern can be observed in central Chile, especially near Santiago. This region, where arachnids have been sampled extensively (Fig. 43A), coincides in our model with the southernmost region with high relative occurrence rate for *Nerudia* (Fig. 43B), but no representative of *Nerudia* has been recorded.

The geographical distribution of *Nerudia* seems to be limited by extreme cold (e.g. higher Andes and southern South America) and/or wet conditions (e.g.

mesic Chaco localities). The climatic limitations observed for *Nerudia*, along with the phylogenetically conserved environmental niche observed for ninetines in general, and the empirical natural history information available, support the hypothesis that *Nerudia* spiders have a limited dispersal ability, even under a strong sampling bias scenario. Eberle *et al.* (2018) suggested that major differences in species richness among Pholcidae clades may be due to evolutionary microhabitat shifts. Ninetinae are extremely conservative in this respect, with most species living under or among rocks and in the dry leaf litter (B. A. Huber & L. S. Carvalho, pers. obs.). This may not only explain why Ninetinae are relatively species-poor compared to other subfamilies, but it also suggests that the diversification of ninetines may be largely due to non-adaptive processes (Dimitrov *et al.*, 2013). The observed phylogenetically conserved environmental niche of ninetines and the similar microhabitat corroborates the higher importance that allopatric processes, instead of dispersal events, have had on the diversification process of Ninetinae (Dimitrov *et al.*, 2013). The phylogenetic niche conservatism shown here was also observed for *Sicarius* Walckenaer, 1847 spiders (Magalhaes *et al.*, 2019). However, these authors emphasized the effect of correlation of geography and climate with the phylogeny of *Sicarius*, hypothesizing that stochastic or deterministic (e.g. unmeasured spatially structured environmental conditions), could be more important for the evolution of that genus (Magalhaes *et al.*, 2019).

The effect of changes in the environmental niche has never been tested for pholcids before. While our results corroborate previous studies on Pholcidae diversification processes (e.g. Dimitrov *et al.*, 2013; Eberle *et al.*, 2018), we admit that they are preliminary in several ways, mainly for being based on a *COI* tree. Thus, upon the availability of more information on the phylogenetic relationships in Pholcidae, including a larger sampling among ninetines, phylogenetic comparative studies should be carried out aiming to disentangle the effect of microhabitat changes, geography and climate autocorrelations with the phylogeny of daddy long-legs spiders.

CONCLUSIONS

- Previously, the Andean genus *Nerudia* Huber, 2000 was poorly known, similar to most Ninetinae genera. It was monotypic and only seven adult specimens from two localities had been reported in the literature. We collected almost 400 adult specimens of *Nerudia* within a month and found them at 24 of the 52 visited localities in northern Argentina.
- We formally describe ten new species based on morphology and *COI* barcodes. Molecular data suggest that our samples contain at least two further species, represented by females only.
- We present the first SEM images of *Nerudia*. These data (mainly tarsal organ shape; also epiandrous spigots and spinnerets) fit the pattern of Ninetinae and thus further confirm the position of *Nerudia* in this subfamily.
- *Nerudia* was mostly found under rocks in arid habitats, at a mean elevation of 1750 m a.s.l. The highest record, at 4450 m a.s.l., represents the highest known record for Pholcidae.
- We present the first karyological data for *Nerudia* and for its putative sister-genus, *Gertschiola*. Karyotype evolution of Ninetinae has been accompanied by a reduction of the number of chromosome pairs. Available data suggest a clade including the South American genera *Gertschiola*, *Nerudia* and *Kambiwa*. The ancestral karyotype of this lineage contained 12 chromosome pairs, a $X_1X_2X_3Y$ or $X_1X_2X_3X_4Y$ system and an X chromosome-linked NOR. The $X_1X_2X_3Y$ system is either a synapomorphy linking *Nerudia* with *Gertschiola* or a more ancestral character, predating the $X_1X_2X_3X_4Y$ system of *Kambiwa*. The complex sex chromosome systems of these spiders are derived from the X_1X_2Y system, which is probably ancestral for Haplogynae spiders.
- We modelled the distribution of *Nerudia*, showing that the genus is not expected to range far beyond the area we sampled. To test this hypothesis, further sampling is needed, in particular in the Atacama and Puna biogeographic provinces, two regions without any record of *Nerudia* so far.
- Our analyses suggest that the environmental niche of *Nerudia*, and that of Ninetinae in general, is phylogenetically conserved. Niche conservatism supports the action of non-adaptive radiation in Pholcidae, in addition to mechanisms such as diversification triggered by evolutionary microhabitat shifts.
- We predict that focused collecting in arid New World regions and habitats will uncover a considerable diversity of Ninetinae, which suffer from disproportionally strong Linnean and Wallacean shortfalls compared to other daddy long-legs spiders.

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AUTHOR CONTRIBUTIONS

BAH: initiation of the project, collecting, taxonomic descriptions, writing. IMAH and JK: karyology, writing. LSC: biogeography, distribution modelling, writing. MAI: permits, collecting, writing. GM: analysis of molecular data, writing.

DATA AVAILABILITY

The *COI* sequences generated by this study have been uploaded to GenBank and can be accessed with the accession numbers in Table 1.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article on the publisher's website.

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