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Arachis microsperma and *A. sesquijuga*: New records of peanut wild relatives for the flora of Paraguay

Arachis microsperma y *A. sesquijuga*: Nuevos registros de parientes silvestres del maní para la flora de Paraguay

Guillermina Macchi Leite[1] & José G. Seijo[2,3]

Summary: As part of a review of wild peanut relatives in Paraguay, new botanical collections were conducted and specimens deposited in various herbaria and databases were re-examined. As an initial outcome of this review, specimens corresponding to *Arachis microsperma* Krapov., W. C. Greg. & Valls from the *Arachis* section and *A. sesquijuga* Valls, L. C. Costa & Custodio, of uncertain sectional location were identified. These two species, previously considered endemic to Brazil, are now added to the flora of Paraguay. Populations of *A. microsperma*, which were previously reported only from southern Mato Grosso do Sul, Brazil, significantly expand the distribution area, primarily within the Amambay and Concepción departments of the Paraguayan territory. Meanwhile, the population of *A. sesquijuga* in Amambay department represents the southernmost record to date for the species.

Key words: *Arachis*, Paraguay, wild peanut.

Resumen: En el marco de una revisión de los parientes silvestres del maní en Paraguay, se realizaron nuevas colecciones botánicas y se re-examinaron los ejemplares depositados en diferentes herbarios y bases de datos. Como resultado inicial de esta revisión, se identificaron especímenes que corresponden a *Arachis microsperma* Krapov., W. C. Greg. & Valls de la sección *Arachis* y *A. sesquijuga* Valls, L. C. Costa & Custodio, cuya asignación a una sección es aún incierta. Estas dos especies, previamente consideradas endémicas para Brasil, ahora se suman a la flora de Paraguay. Las poblaciones de *A. microsperma,* que hasta el momento eran reportadas solo del sur de Mato Grosso do Sul, amplían significativamente el área de distribución de la especie, ubicándose en su mayor parte en los departamentos Amambay y Concepción del territorio paraguayo. Por otro lado, la población de *A. sesquijuga* de Amambay se constituye en la más austral registrada hasta la fecha para la especie.

Palabras clave: *Arachis,* maní silvestre, Paraguay.

Introduction

The genus *Arachis* is native to South America and comprises 84 recognized species (Krapovickas & Gregory, 1994; Valls & Simpson, 2005, 2017; Valls *et al.*, 2013; Santana & Valls, 2015; Seijo *et al.*, 2021, 2025). Currently, *Arachis* species range from the southern Amazon to La Plata river and from the foothills of the Andes at 1,450 meters above sea level to the Atlantic coast. It has been postulated that the genus originated

[1] Facultad de Ciencias Agrarias, Universidad Nacional de Asunción (FCA, UNA), Campus Universitario, San Lorenzo, Paraguay. E-mail: guille.macchi.gml@gmail.com
[2] Instituto de Botánica del Nordeste (IBONE, UNNE-CONICET), Facultad de Ciencias Agrarias, Campus Cabral, Corrientes, Argentina. E-mail: jgseijo@yahoo.com
[3] Departamento de Biología, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Campus Deodoro Roca, Corrientes, Argentina.

in the Amambay Mountains, on the border between Mato Grosso do Sul State (Brazil) and Northeastern Paraguay, where species with characters considered more ancestral to the genus are found, all belonging to the *Trierectoides* Krapov. & W. C. Greg. section (Krapovickas & Gregory, 1994).

In the most recent taxonomic works on the genus, 15 wild species have been recognized for Paraguay, corresponding to five sections (Krapovickas & Gregory 1994; Valls & Simpson, 2005). These include *A. guaranitica* Chodat & Hassl. (sect. *Trierectoides*); *A. major* Krapov. & W. C. Greg. and *A. paraguariensis* Chodat & Hassl. subsp. *paraguariensis* from sect. *Erectoides* Krapov. & W. C. Greg.; *A. lignosa* (Chodat & Hassl.) Krapov. & W. C. Greg., *A. pflugeae* C. E. Simpson, Krapov. & Valls, and *A. hassleri* Krapov., Valls & C. E. Simpson from sect. *Procumbentes* Krapov. & W. C. Greg.; *A. pseudovillosa* (Chodat & Hassl.) Krapov. & W. C. Greg., *A. glabrata* Benth. var. *glabrata*, *A. glabrata* Benth. var. *hagenbeckii* (Harms ex Kuntze) F. J. Herm. and *A. nitida* Valls, Krapov. & C. E. Simpson from sect. *Rhizomatosae* Krapov. & W. C. Greg. series *Rhizomatosae*; and *A. batizocoi* Krapov. & W. C. Greg., *A. duranensis* Krapov. & W. C. Greg., *A. correntina* (Burkart) Krapov. & W. C. Greg., *A. cardenasii* Krapov. & W. C. Greg., *A. diogoi* Hoehne, and *A. schininii* Krapov., Valls & C. E. Simpson from sect. *Arachis*. The species in the later section constitute the secondary gene pool of cultivated peanut, and others are important as natural forages, especially those from the *Rhizomatosae* section.

Despite the recognition of these species, Paraguay remains underexplored in terms of genetic resources. Biogeographical analyses of the genus based on collections predating 2000 have shown that the states of Mato Grosso and Mato Grosso do Sul in Brazil are the most important diversity center for the genus (Jarvis *et al.*, 2003). The collections made in Bolivia since 2001 have identified the Chiquitanía and Chaco regions of Bolivia as another important diversity center for the genus, mainly for the *Arachis* and *Procumbentes* sections (Seijo *et al.*, 2012). However, Paraguay still appears as a significant gap of diversity, once it is geographically adjacent to the two mentioned centers and encompasses the potential center of origin of the genus.

For this reason, exploratory expeditions were conducted in the northeastern Paraguay in 2020 to increase the collections of *Arachis*. Specimens from various herbaria and databases, particularly those collected after the publication of the genus monograph (Krapovickas & Gregory, 1994), were also re-analyzed. The objective of this study is to present two new additions to the flora of Paraguay, and to provide species descriptions incorporating the variability of the Paraguayan specimens.

Materials and Methods

Biological material

Given that *Arachis* specimens collected in Paraguay since 1994 were mainly deposited in herbaria of Paraguay and Argentina, with duplicates in herbaria of United States and Switzerland, a physical inventory of wild specimens from Paraguay was conducted at the CTES Herbarium "Dra. Carmen L. Cristóbal", of Instituto de Botánica del Nordeste (IBONE), CONICET, UNNE, Corrientes, Argentina and of Facultad de Ciencias Químicas (FCQ), Universidad Nacional de Asunción (UNA), San Lorenzo, Paraguay. Public domain databases such as SpeciesLink (https://specieslink.net/), Conservatoire & Jardin Botaniques de la Ville de Genève (http://www.ville-ge.ch), Missouri Botanical Garden (https://www.tropicos.org/collection/search), Museum of Natural History in Paris (https://science.mnhn.fr/institution/mnhn/search), and Peanut Base (https://peanutbase.org/germplasm/map) were also consulted.

Collection trips were conducted under the regulations of Paraguay's Ministerio del Ambiente y Desarrollo Sostenible (MADES), with approval from the native wild flora scientific research project titled Colecta de germoplasma silvestre de *Arachis* de Paraguay, permit No. 123/2019. The work was carried out under the project Recursos genéticos de maní silvestre y cultivado de Paraguay (Resolution FCA, UNA 1094-00-

2019), within the framework of the doctoral thesis: Caracterización morfológica y molecular de germoplasma silvestre de *Arachis* de Paraguay, UNNE, Corrientes, Argentina. The expeditions took place from January to March 2020, mainly in the departments of Central, Paraguarí, Misiones, Ñeembucú, Amambay, and Concepción. Herbarium specimens were prepared from these materials and live plants are maintained under greenhouse cultivation at the Facultad de Ciencias Agrarias, Universidad Nacional de Asunción.

Taxonomic identification

Preliminary taxonomic identification of the species was conducted in the field and by following standard procedures in the laboratory using the genus monograph (Krapovickas & Gregory, 1994) and complementary works (Valls & Simpson, 2005; Valls *et al.*, 2013).

Morphological analysis

Morphological analysis was carried out on herbarium specimens from Paraguay corresponding to historical collections deposited in the CTES herbarium, and those from the new expedition in 2020.

To corroborate the identity of the materials collected in Paraguay, a qualitative and quantitative analysis was performed, comparing them with available specimens of similar morphology collected in Brazil.

For measurements, three replicates were considered for each evaluated material. Quantitative characters measured included lengths of the fused and free portions of the stipule; length of petiole and rachis; length, width, and length/width ratio of the distal and proximal pairs of leaflets; length and width of fruit segments and seeds; length of the hypanthium and the upper lip of the calyx. Qualitative characters included hairs and bristles on the stipule, petiole, rachis, pulvinus, epiphyll, hypophyll, margin, central vein, hypanthium, and calyx; corolla color; leaf shape; presence of beak in the fruit segment, reticulum and ribs in the exocarp, soil retention of the fruit; presence of beak in the seed. All vegetative measurements were taken on organs of lateral branches since no examined specimen presented a central axis.

Data analysis

Descriptive statistics, including mean, minimum, and maximum values were calculated for quantitative variables. Principal component analysis (PCA) and cluster analysis were performed using standardized data and Gower's distance, with the average linkage hierarchical clustering procedure (UPGMA). Data were analyzed using the Infostat program (Di Rienzo *et al.*, 2020).

Results

During the review of *Arachis* herbarium specimens from Paraguay at the CTES herbarium, five exsiccates (*Silva et al.*, *3776*, *3784*, *3806*, *3817* and *Dematteis et al.*, *2920*) and one material (*Simpson et al. 3941*) in the PeanutBase/Germplasm Map database were identified as *A. microsperma* Krapov., W. C. Greg. & Valls. During collection trips, three populations *Macchi & Seijo 78*, *111*, and *113* were found that exhibited characteristics consistent with this species. Principal component analysis indicated that the Paraguayan samples covered most of the species' variability range, with one Brazilian specimen sharing characteristics with those from Paraguay and the other occupying a morphological extreme of the species. These findings significantly extend the geographical area where *A. microsperma* inhabits, highlighting that the majority of the species distribution is in Northern Paraguay, east of the homonymous river.

The materials exhibited a wide range of variability in various quantitative and qualitative characters, especially in the distribution and type of pubescence in different organs. The accessions plotted on CP1 and CP2 in the PCA analysis are presented in Fig. 1 A. The characters with the most significant descriptive value in CP1 and CP2, which accounts for 52.2% of the total variation and have a cophenetic correlation of 0.967, are presented in Table 1. In general, specimens from Mato Grosso do Sul and those from the Amambay department, near the Apa river, showed smaller dimensions in all organs than those from the Concepción department.

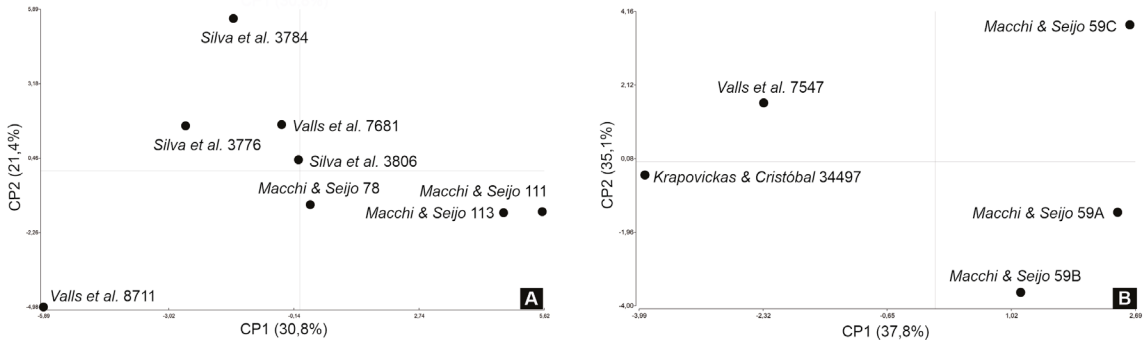

Fig. 1. Principal Component Analysis (PCA) of morphological variables in *Arachis* species new to the flora of Paraguay. A: *A. microsperma*, eight analyzed herbarium specimens are plotted: *Macchi & Seijo 78*, *111* and *113*, *Silva et al. 3776*, *3784* and *3806*, are from Paraguay and *Valls et al. 7681* and *8711* are from Brazil. B: *A. sesquijuga* and *A. tuberosa*, five analyzed herbarium specimens are plotted: *Macchi & Seijo 59A*, *B*, and *C* are from Paraguay (*A. sesquijuga*) and *Valls et al. 7547* and *Krapovickas & Cristóbal 34497* are from Brazil (*A. tuberosa*).

During exploratory expeditions in the Amambay department, specimens with erect stems and trifoliolate leaves were also collected. Initially, these specimens were identified as *A. tuberosa* Bong. ex Benth. due to the absence of a rachis in many of their leaves. However, in nearly half of the leaves they exhibit a very short rachis (< 1.8 mm), and also obovate leaflets, traits that are inconsistent with *A. tuberosa*. Recently, another trifoliolate species, distinct from *A. guaranitica* due to its non-linear leaflets, was described from Mato Grosso do Sul, Brazil (Valls *et al.*, 2013). This species, named *A. sesquijuga* Valls, L. C. Costa & Custodio, has imparipinnate leaves with a rachis ranging between 1.6-4.3 mm in length. Although the trifoliolate specimens from Paraguay do not completely match the rachis length range of *A. sesquijuga*, they show other characteristics, such as plant size, fruit production, and hair distribution (Valls, J. F. M., 2024, pers. communication), that make them more compatible with *A. sesquijuga*.

Table 1. Variables with major descriptive values in the CP1 and CP2 of PCA analyses of *A. microsperma* and *A. sesquijuga.*

A. microsperma			***A. sesquijuga* and *A. tuberosa***		
Variables	**CP 1**	**CP 2**	**Variables**	**CP 1**	**CP 2**
Branch length	0.40	**-0.76**	Stipule: Length of the free part	-0.26	**0.91**
Stipule: Length of the fused part	**0.83**	-0.16	Length of the rachis	**0.90**	0.11
Stipule: Length of the free part	**0.84**	-0.49	Length of the distal leaflet	-0.40	**0.91**
Ratio of the length and width of the distal leaflet	**0.90**	-0.24	Width of the distal leaflet	-0.01	**0.99**
Ratio of the leng/width of the proximal leaflet	**0.88**	-0.27	Length of the proximal leaflet	-0.37	**0.91**
Long hairs on the blade of the free stipule	-0.17	**0.72**	Width of the proximal leaflet	-0.12	**0.99**
Short hairs on the hypanthium	-0.15	**0.68**	Short hairs on the hypophyll of the leaflet	**0.78**	0.38
Long hairs on the hypanthium	0.26	**-0.68**	Long hairs on the margin of the leaflet	**0.96**	-0.09
Width of the fruit segment	-0.21	**0.78**	Bristles on the margin of the leaflet	**-0.96**	0.09
Depth of the fruit segment	**0.89**	-0.03	Long hairs on the midrib of the leaflet	**0.96**	-0.09

This taxonomic approach was complemented with a morphological analysis using principal components, including the materials from Paraguay together with *A. tuberosa* from Brazil. Accessions are projected on CP1 and CP2 of the PCA analysis in Fig. 1 B. Table 1 presents characters with the most significant descriptive value in CP1 and CP2, which accounts for 72.9% of the total variation and have a cophenetic correlation of 1. The record of *A. sesquijuga* in the Amambay department constitutes the southernmost locality for the species and is a new citation for Paraguay.

Below, we present a description of the species cited as novelties for Paraguay, with measurements corresponding to the studied specimens from the country.

Arachis microsperma Krapov., W. C. Greg. & Valls (Section *Arachis*) (Fig. 2 A-F).

Perennial plant with a taproot. Prostrate branches with anthocyanin pigmentation. Stipule with a fused portion 4.99 (4.28-6.17) mm long and the free portion 9.85 (6.65-13.53) mm long, glabrous, rarely with few short hairs on the fused portion and long ones on the free portion, occasionally with bristles, margin with few long hairs. Petiole 14.53 (5.22-32.79) mm and rachis 4.95 (2.92-6.35) mm long. Tetrafoliate leaves, lanceolate to linear-lanceolate leaflets. Distal leaflet 13.76 (8.75-19.52) × 6.52 (5.41-8.59) mm, with a length/width ratio of 2.16 (1.50-3.33). Proximal leaflet 12.15 (7.97-15.94) × 5.55 (4.53-7.40) mm, with a length/width ratio of 2.22 (1.73-3.10). Petiole and rachis glabrous or with short and long hairs, rarely with few bristles on the rachis; pulvini with sparsely short hairs (rarely without them) and densely long hairs (rarely sparsely). Epiphyll glabrous, rarely with sparsely short and long hairs, hypophyll with sparsely short hairs, rarely with sparsely long hairs, midrib with sparsely long hairs, margin with sparsely long hairs, rarely with bristles. In the flowers, hypanthium 56.7 (25.7-75.0) mm long, with sparsely short and long hairs. Upper lip of the calyx 5.55 (5.17-6.28) mm long, with sparsely short and long hairs, with very dense bristles. Standard salmon-colored on its outer surface, light orange to orange on the inner surface, yellow in the proximal part, rarely with red lines on the front surface; wings yellow. Pegs glabrous, rarely with sparsely long hairs. Fruit segment 9.30 (8.70-10.12) mm long, 4.94 (4.85-5.94) mm wide, and 4.38 (3.64-5.30) mm deep, with a moderately to very prominent beak and moderate to prominent reticulum. The seed purple or brown-colored, 7.75 (6.77-8.74) mm long, 3.30 (3.14-3.46) mm wide, and 3.95 (3.28-4.62) mm deep, with a prominent beak.

Geographic distribution: Until the present study, *A. microsperma* had only been reported from southwest of the Mato Grosso do Sul state, Brazil, near Bela Vista city (Krapovickas & Gregory, 1994). Here we add another specimen from Brazil, collected near the Capivara stream some 40 km East of Porto Murtinho city. More important is that the distribution of the species is greatly extended toward two departments in Paraguay, namely Amambay (in the Bella Vista Norte District near the Apa river) and Concepción (in the San Carlos and Concepción districts). In Concepción department, it is primarily found in the Humid Chaco ecoregion, while in Amambay, it occurs in the riverbanks of the Apa river with components of the Humid Chaco and Cerrado.

Studied material: **BRASIL**. **Mato Grosso do Sul**: Mun. Bela Vista, junto à alfândega de Bela Vista, em gramados ornamentais, área urbanizada próxima à margem do rio Apa, 22-IV-1984*, Valls et al. 7681* (isotype CTES); entrada de Bela Vista desde Jardim, 24-IV-1985, *Valls et al. 8711* (paratype CTES); Mun. Porto Murtinho, Córrego Capivara, 17-III-1985, *Hatschbach & Zelma 49211* (CTES). **PARAGUAY. Amambay**: Distrito Bella Vista Norte, camino San Carlos a Puentesinho, km 37, 22°22'43''S, 56°36'47''W, 26-I-1997, *Silva et al. 3784* (CTES); a 8,5 km del puente en Bella Vista, en la carretera a San Carlos, 0,5 km más allá del primer riachuelo, 22°10'48''S, 56°31'12''W, 27-IV-2007, *Simpson et al. 3941* (base de datos Peanut Base); próximo al río Apa, cerca del puente que cruza a la frontera con Brasil, 22°06'55.3''S, 56°31'11.4''W, 172

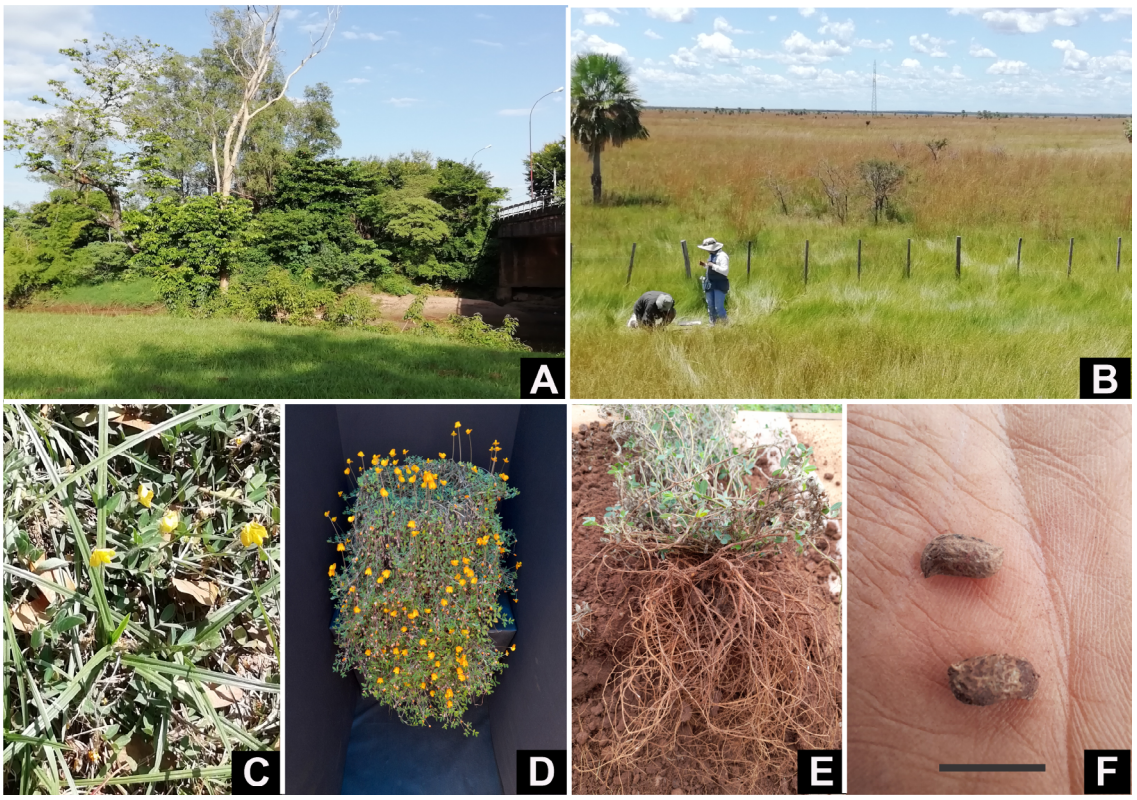

Fig. 2. *Arachis microsperma*. A: Environment of the species sandbanks of Apa river with open patches of gallery forest. B: Environment of the species open field with "caranday" palm in Humid Chaco, in the road from Vallemí to Concepción. C: Plant at the collection site. D: Plant conserved *ex situ* in greenhouse at FCA, UNA. E: Root system in the greenhouse. F: Fruit harvested from plants cultivated in greenhouse. Scale bar = 1 cm.

m, 01-III-2020, *Macchi & Seijo 78* (CTES, FCQ). **Concepción**: Distrito San Carlos, camino San Carlos a Puentesinho, km 32,3, 22°18'56''S, 57°10'05''W, 26-I-1997, *Silva et al. 3776* (CTES); Distrito Concepción, camino río Aquidabán a Puerto Vallemí Hu, km 14, 22°57'12''S, 57°21'24''W, 31-I-1997, *Silva et al. 3806* (CTES); Colonia Primavera, 23°22'38''S, 57°24'57''W, 02-II-1997, *Silva et al. 3817*; 4,3 km E de Concepción, camino a Yby Yau, 23°24'19''S y 57°23'00''W, 29-04-2008, *Dematteis et al. 2920* (CTES); km 61 camino de Vallemí a Concepción, 22°55'35.1''S, 57°21'53.5''W, 106 m, 04-III-2020, *Macchi & Seijo 111* (CTES, FCQ); en el paseo central de la doble avenida de Concepción, camino de Vallemí-Concepción, 23°23'23.6''S, 57°25'21.3''W, 84 m, 04-III-2020, *Macchi & Seijo 113* (CTES, FCQ).

Obs.: The herbarium specimens from Paraguay included for morphological description were *Silva et al. 3776*, *3784* and *3806*, *Macchi & Seijo 78*, *111*, and *113*. Additionally, measurements of the Brazilian materials *Valls et al. 7681* and *8711* were included for principal component analysis.

Arachis sesquijuga Valls, L. C. Costa & Custodio (Section yet undefined) (Fig. 3. A-F).

Perennial plant, with branched tuberous roots. Erect stems 39.4 (11.2-65.5) cm in length, exhibiting anthocyanin pigmentation. Stipules with the fused part 12.62 (9.89-15.94) mm long, and the free part 15.5 (10.27-20.89) mm long, surface with sparse short hairs in the fused and free portions and margin with

very dense long hairs. Petiole 6.06 (5.47-6.40) mm, and rachis 1.30 (0-1.89) mm long. Leaves trifoliolate, with a wide obovate distal leaflet and narrow obovate proximal ones. The distal leaflet 32.81 (27.94-41.03) × 12.32 (9.26-16.62) mm, with a length/width ratio of 2.73 (2.48-3.02). Proximal leaflets 28.73 (24.46-35.76) × 10.26 (7.74-13.48) mm, with a length/width ratio of 2.85 (2.68-3.15). Petiole with sparse short hairs and very dense long hairs, rachis and pulvini with very dense long hairs. Epiphyll and hypophyll with sparse short hairs or glabrous. Margin of leaflets with sparse short hairs and very dense long hairs. Midvein with dense long hairs. In the flower, hypanthium 33 mm long, with sparse long hairs; calyx with sparse short hairs, with upper lip 4.5 mm long. Standard light orange with a yellow proximal portion, with red lines in the front surface, wings yellow. Pegs with sparse short and long hairs. Fruit segment with a very prominent beak and visible ribs, 13.81 (13.11-14.81) mm long, 5.6 (4.72-6.67) mm wide, and 6.19 (5.62-7.076) mm deep. Seed purple, with a prominent seed beak, 10.89 (10.30-11.27) mm in length, 4.19 (4.08-4.45) mm in width, and 4.45 (3.82-5.28) mm deep.

Geographic distribution: *Arachis sesquijuga* is primarily found in Mun. Sidrolândia Mato Grosso do Sul State, Brazil, is known only from the type locality, in areas of red soil with open cerrado grasslands, now highly disturbed by agricultural use with sugarcane, soybeans, maize, cotton and exotic grass pastures (Valls *et al.*, 2013). In Paraguay, *A. sesquijuga* was found at a single site in the Department of Amambay, Pedro Juan Caballero District, at

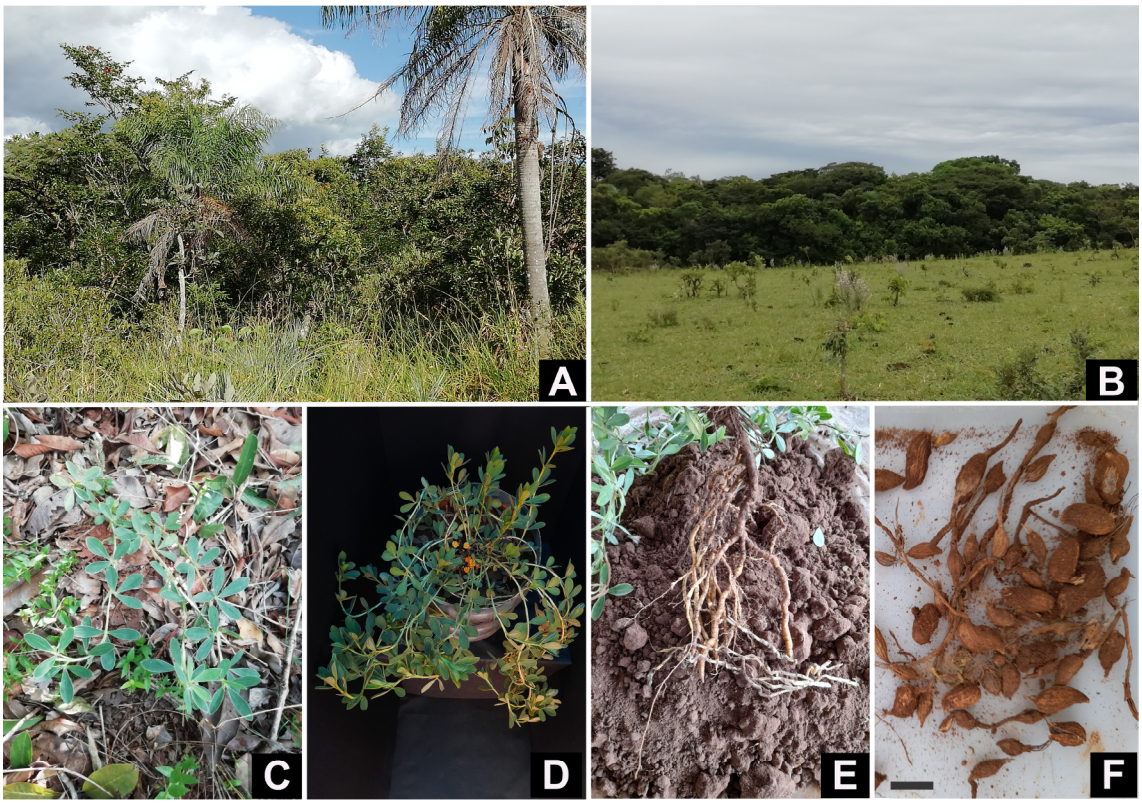

Fig. 3. *Arachis sesquijuga*. A: Environment of specimen *Macchi & Seijo 59A* in Cerrado. B: Environment of specimen *Macchi & Seijo 59B* in open grassland with *Brachiaria*, at the edge of a large island of forest. C: Plant at the collection site. D: Plant conserved *ex situ* in greenhouse at FCA, UNA. E: Root system in the greenhouse, notice their enlargements. F: Fruit with ribs and pronounced beak harvested from plants cultivated in greenhouse. Scale bar = 1 cm.

Colonia Estrella, in an anthropized cerrado. This location borders Mato Grosso do Sul, Brazil. Population remnants were found in an area with intensive agricultural management, under bushes in cattle grazing pasture and near a soybean field.

Studied material: **PARAGUAY**. **Amambay**: Distrito Pedro Juan Caballero, Colonia Estrella, debajo de una *Butia* sp., 22°17'55.313"S, 55°50' 26.128"W, 694 m, 25-II-2020, *Macchi & Seijo 59A* (CTES, FCQ); *ibid.*, al borde de una isleta de monte alto, en cerrado, intervenido con *Brachiaria* y sobrepastoreo, 22°18'2.722"S, 55°50'23.122"W, 688 m, *Macchi & Seijo 59B* (CTES, FCQ); *ibid.,* campo abierto, cerrado con *Brachiaria humidicola* al borde de una plantación de soja, 22°18'9.133" S, 55°50'13.635"W, 694 m, *Macchi & Seijo 59C* (CTES, FCQ).

Obs. 1: Both *A. tuberosa* and *A. guaranitica* have trifoliolate leaves, with ternate leaflets that insert at the tip of the petiole at the same height, without a rachis (Krapovickas & Gregory, 1994; Valls *et al*., 2013). These species differ from *A. sesquijuga* because the latter has a rachis that can reach up to 4.6 mm, separating the insertion of the distal leaflet from the insertion of the basal ones at the tip of the petiole. The specimens from Paraguay exhibit leaves that were variable in this character, some lacking a rachis and others having a rachis up to 1.8 mm long. Since this variation was observed even in a single plant, all the specimens collected in Paraguay were assimilated to *A. sesquijuga*.

Obs. 2: Although the specimens from Paraguay do not completely match the described rachis range of *A. sesquijuga*, other characters differentiate them from *A. tuberosa.* Below are some distinguishing characters provided by J. F. M. Valls during the revision of this manuscript (Valls, J. F. M., 2024, pers. comm.). *Arachis tuberosa* forms small bushes with a few erect stems, sometimes, with a narrow base and a vertical root that becomes tuberous a few centimeters below, and remains cylindrical to globose. The leaflets are of similar size, usually without hairs, with only a few scattered setae in their margin. These plants produce very rare seeds each year; the pegs are not visible above ground, since the flower buds develop underground, where the ovary remains as the hypanthium of the flower expands.

By contrast, *A. sesquijuga* has both low and higher branches above ground, the bushes have a wider base and resemble some species of the *Erectoides* section. They have a branched root system with tuberosities of various shapes. The leaves are pinnate-trifoliolate, the basal leaflets are broader, possessing distinct marginal hairs and setae. It produces many pegs above the soil surface and yields numerous seeds. Based on these descriptions, the specimens from Paraguay are more compatible with *A. sesquijuga*.

Obs. 3: The herbarium specimens from Paraguay used for morphological characterization were *Macchi & Seijo 59A*, *59B*, and *59C*. For principal component analysis, the specimens *Krapovickas & Cristóbal 34497* and *Valls et al. 7547* of *A. tuberosa* from Brazil were added.

Obs. 4: The nuclear ITS1-5,8S-ITS2 region of the 45S ribosomal genes of the *Macchi & Seijo* 59 specimen was compared to those of *A. tuberosa* and *A. sesquijuga* (Moretzohnn *et al*., unpublished). The sequence of the material analysed here showed the highest similarities with those of the accession *Valls et al.* 15563 of the latter species.

Final considerations

This study supports the hypothesis that Paraguay harbors a more diverse array of *Arachis* species than previously documented in the existing literature. The expanded collections in previously underexplored areas, coupled with the analysis of new materials, contributed to enrich the inventory of wild *Arachis* relatives in Paraguay. With these two new citations, the number of wild *Arachis* species present in Paraguay rises to 17.

From an agronomic perspective, the collected materials and the information generated

from the characterization of available wild *Arachis* accessions are important for the *in situ* preservation of genetic resources and the establishment of *ex situ* collections that can be utilized in peanut improvement programs.

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