

# *Waltheria marielleae* (Byttnerioideae, Malvaceae), a new species from north-eastern Brazil supported by morphological and phylogenetic evidence

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## Abstract

**Background** – *Waltheria marielleae* is a new species of Malvaceae endemic to north-eastern Brazil that occurs only in the states of Pernambuco and Alagoas, in areas of Atlantic Forest and Caatinga. It is characterized by cinereous leaves, axillary and sessile to subsessile inflorescences, pallid yellow corollas with apically eciliate petals and fan-plumose stigmas. **Material and methods** – DNA was extracted from leaf tissue and the markers *matK*, *ndhF*, and ITS were amplified using universal primers, with PCR products purified and sequenced. Phylogenetic analysis was performed, including DNA

universal primers, with PCR products purified and sequenced. Phylogenetic analysis was performed, including DNA sequences obtained from GenBank. Morphological studies were based on the analysis of specimens deposited in seven herbaria and specimens collected in Pernambuco state, Brazil.

**Results** – *Waltheria marielleae* is morphologically and phylogenetically related to pantropical *W. indica* and *W. ackermanniana*, with these three species forming a well-supported clade. Overall, phylogenetic molecular analysis suggests the monophyly of *Waltheria*, with the two currently proposed sections also being monophyletic, and *Melochia* as its sister group. The new species is assessed here as Endangered, according to IUCN criteria. In addition, we formally present new occurrences of *W. ackermanniana* and *W. rotundifolia*.

**Conclusion** – In this study, a complete morphological description, illustration, distribution map, and phylogenetic tree are provided for *Waltheria marielleae*. This species is compared with morphologically and phylogenetically related species (*W. ackermanniana, W. indica*, and *W. rotundifolia*) and an identification key to the species occurring in Alagoas and Pernambuco is provided.

## **Keywords**

Alagoas, endangered species, endemism, fan-plumose stigmas, Hermannieae, Pernambuco, phylogenetic placement

## INTRODUCTION

The genus *Waltheria* L. belongs to Malvaceae, subfamily Byttnerioideae (Alverson et al. 1999; Whitlock et al. 2001). With approximately 60 species particularly distributed in tropical regions, Brazil is its main centre of diversity and endemism, harbouring 32 species, 23 of which are endemic to the country (Coutinho et al. 2020b), and Mexico is the second centre with 15 species and nine endemics (Saunders 1993; Saunders 2007). The north-eastern region of Brazil is the second most diverse area in terms of number of species, although only three are endemic: *Waltheria saundersiae* T.S.Coutinho, *W. selloana* K.Schum., and *W. rotundifolia* Schrank (Coutinho and Alves 2019; Coutinho et al. 2020b). Surveys focusing on *Waltheria* in north-eastern Brazil are scarce, however, the genus has been treated in some local taxonomic studies (Cristóbal et al. 1995; Amorim et al. 2009; Alves et al. 2011; Amorim 2012). Detailed morphological investigation of these species often results

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in taxonomic novelties, as for example in Coutinho and Alves (2019), in which *W. saundersiae*, a species endemic to the state of Bahia, is described, and in Coutinho et al. (2020a), which re-established the name *W. brachypetala* Turcz., a widespread species in the north-eastern region with a few collections in the state of Minas Gerais (southeastern region), previously considered a synonym of *W. ferruginea* A.St.-Hil. In addition, *W. coriacea* J.G.Saunders was recently described with occurrences in north-eastern Brazil (Saunders 2021).

Although *Waltheria* is a diverse group, a molecular phylogenetic reconstruction of the genus is lacking. Whitlock et al. (2001) conducted a molecular phylogeny of Byttnerioideae based on the plastid gene *ndhF*, however, only one unidentified species of *Waltheria* was sampled and no inference could be drawn. Richardson et al. (2015) also sampled one species, *W. madagascariensis* Hochr., as the outgroup of a molecular phylogeny of *Theobroma* L.

During a taxonomic study of Brazilian *Waltheria* performed by the first author, a new species was found in herbarium collections and later re-collected in the state of Pernambuco. This new taxon shares morphological features with the pantropical *Waltheria indica* L. and the disjunct *W. rotundifolia*, which occurs in Mexico and Brazil. Because of the complexity and homogeneity of the morphological characters shared by the species of the genus, we aim to place the new species of *Waltheria* in a phylogenetic context, and investigate whether morphologically related species represent a monophyletic group.

## MATERIAL AND METHODS

#### Taxa sampling

We sampled 13 taxa belonging to the family Malvaceae, nine of them species of *Waltheria: W. ackermanniana* K.Schum., *W. brachypetala* Turcz., *W. cinerascens* A.St.-Hil., *W. indica*, *W. lantanaefolia* A.St.-Hil., *W. macropoda* Turcz., *W. rotundifolia*, *W. saundersiae*, and the new species. *Byttneria aculeata* (Jacq.) Jacq., *Commersonia fraseri* J.Gay, *Melochia corchorifolia* L., and *Theobroma cacao* L. were used as outgroup and their sequences were obtained from GenBank. The internal transcribed spacer (ITS) of the nuclear ribosomal DNA and the plastid markers *ndhF* and *matK* were used for the phylogenetic analysis (Supplementary file 1).

#### DNA extraction, amplification, and sequencing

Total DNA was extracted from 0.3 g of silica dried leaf tissue using the protocol from Doyle and Doyle (1987), with modifications from Ferreira and Grattapaglia (1996). The markers *matK* (Sang et al. 1997), *ndhF* (Olmstead and Sweere 1994), and ITS (White et al. 1990) were amplified using universal primers. Polymerase chain reaction (PCR) was carried out in a total volume of 50  $\mu$ L, containing 50

ng of gDNA, 2 mM MgCl<sub>2</sub>, 0.1 mM dNTPs, 1× PCR buffer, 1  $\mu$ L DMSO, 0.4  $\mu$ M of each primer, and homemade Taq polymerase. PCR amplification consisted of one initial denaturing step at 95°C for 5 min, followed by 35 cycles of 1 min at 95°C, 1 min at annealing temperature (50°C for ITS, 52°C for *matK*, 50°C for *ndhF*), and 1 min at 72°C, with a final extension of 10 min at 72°C.

PCR products were purified by ethanol precipitation: 0.1 vol of 3 M sodium acetate and 2 vol of ethanol were added and then centrifuged. Sanger sequencing was performed at the Sequencing Platform of LABCEN/CB/UFPE (Brazil). All 22 newly obtained sequences are available at GenBank (Supplementary file 1).

#### **Phylogenetic analyses**

We used jModelTest v.2.1.6 to assess the best DNA substitution model for each individual marker (Darriba et al. 2012) based on the Akaike Information Criterion (Akaike 1974). The best fitting model for the three regions was GTR+G (Supplementary file 2). Phylogenetic relationships were inferred using the Bayesian Inference (BI) approach implemented in MrBayes v.3.2.6. (Ronquist et al. 2012). Analyses were performed for each region separately and all concatenated. Four independent runs with four Markov Chain Monte Carlo (MCMC) runs were conducted, sampling every 1,000 generations for 10,000,000 generations. Each run was evaluated in TRACER v.1.6 (Rambaut et al. 2014) to determine if the estimated sample size (ESS) for each relevant parameter was higher than 200 and a burn-in of 25% was applied. The majority rule consensus tree and posterior probability (PP) were visualized and edited in FigTree v.1.4.2. (Rambaut 2014). All BI and jModelTest analyses were performed in the CIPRES Science Gateway (Miller et al. 2010).

#### Morphological description

Morphological studies were based on the analysis of specimens housed at HPISF, HUEFS\*, IPA, MAC, NY\*, PEUFR, UEC, and UFP (acronyms follow Thiers continuously updated; \*digital images), in addition to material collected by the first author in Bonito, Pernambuco, Brazil. Morphological terminology follows Radford et al. (1974) and Harris and Harris (2001) for general characters, including indumentum, Webster et al. (1996) for trichome type, and Saunders (1993) for stigma morphology. Morphological description follows previous studies by the first author (Coutinho and Alves, 2019, 2020; Silva-Coutinho et al. 2019; Coutinho et al. 2020a).

#### **Conservation status and distribution map**

Conservation status was assessed with support of the GeoCat tool (Bachman et al. 2011), with Area of Occurrence (AOO) based on a 2-km cell width and classified according to IUCN (2019) criteria. The distribution map was produced using QGIS v.3.0.1 (QGIS Development Team 2018), with coordinates obtained from herbarium specimen labels or georeferenced with Google Earth.

## **RESULTS AND DISCUSSION**

# Phylogenetic relationships in *Waltheria* and placement of the new species

We present the first phylogenetic analysis of the genus *Waltheria* to date (Fig. 1), allowing inferences on some species relationships. In the consensus tree of the concatenated analysis, containing one nuclear and two plastid regions, *Waltheria* was recovered as monophyletic (PP 0.98) with *Melochia* as its sister group (PP 1), as suggested by Alverson et al. (1999) and Richardson et al. (2015). *Waltheria macropoda*, the only species of *W*. sect. *Stegowaltheria* K.Schum. in our analysis, emerged as sister to the rest of the species. This species is characterized

by wide stipules with rounded base, branches with only simple trichomes, capsules with operculate dehiscence, and verrucose seeds. The remaining species represent W. sect. Waltheria, characterized by narrow stipules with truncate base, branches with stellate trichomes, capsule with loculicidal dehiscence, and smooth to slightly crenulate seeds. Waltheria brachypetala is the only sampled species with multiradiate trichomes on the branches, in addition to the clavate stigmas, and capsules with rounded apex, emerging as sister to the remaining species in this clade. Waltheria rotundifolia is sister to two well-supported clades with three species each, both sharing the fan-plumose stigmas and capsules with truncate apex. However, W. rotundifolia differs by the capsule with tomentose apex and total dehiscence, opening in two valves.

Waltheria marielleae emerged in a well-supported clade (PP 0.99) with *W. indica* and *W. ackermanniana*. These three species share narrowly triangular stipules, bracteoles longer than the calyx, fan-plumose stigmas, and capsules with sericeous apex. Although *W. marielleae* 



**Figure 1.** Phylogenetic relationships of *Waltheria* generated by Bayesian inference based on concatenated nuclear (ITS) and plastid (*matK* and *ndhF*) regions. The bar with horizontal lines represents *W.* sect. *Stegowaltheria*, and with vertical lines, *W.* sect. *Waltheria* sensu Schumann (1886). Numbers above the branches are PP values. The new species, *W. marielleae*, is highlighted in bold.

is morphologically more closely related to *W. indica* by sharing trichome type on the branches, stipule shape, inflorescence position, stigma morphology, and characters related to the capsules, it appeared as sister to the *W. indica* + *W. ackermanniana* clade. The new species differs from the other two by the acute lobe apex calyx and eciliate petals. *Waltheria marielleae* and *W. rotundifolia* are morphologically related, but our analysis shows they are not phylogenetically close. Morphological affinities are shown below and in Table 1.

## TAXONOMIC TREATMENT

*Waltheria marielleae* T.S.Coutinho & M.Alves, **sp. nov.** urn:lsid:ipni.org:names:77305493-1 Figs 2A–F, 3

**Type.** BRAZIL • Pernambuco, Mun. Bonito, Reserva Municipal de Bonito; 13 Jun. 2018; fl., fr.; *T.S. Coutinho et al.* 375; holotype: UFP; isotypes: BHCB [207616], CEN, EAC, HCDAL, HUEFS, JPB, MAC [0065667], MBM, RB, SPF, UFMT, VIES.

**Diagnosis.** *Waltheria marielleae* resembles *W. indica* by the branch indumentum, narrowly triangular stipules, elliptic leaf blades, axillary inflorescences, and fanplumose stigmas, but differs by the olive green to greyish-green leaf blades (vs greenish), larger and distylous flowers (vs smaller and homostylous flowers), acute calyx lobe apex (vs acuminate), and spatulate and eciliate petals (vs oblong and ciliate).

Description. Herbs to shrubs 0.7-1.5 m tall. Branches terete, apically compressed, sericeous, trichomes stellate, sessile or subsessile, whitish; bark sparsely lenticellate, lenticels verrucose. Stipules  $6.5-8 \times 0.8-1$  mm, narrowly triangular, base truncate, margins entire, sparsely ciliate, apex acuminate, adaxial surface pubescent, trichomes simple and stellate, sessile, abaxial surface pubescent, trichomes stellate, sessile; vein 1, conspicuous, prominent abaxially. Leaves simple, alternate, spirally arranged along the branches; petiole 1.0-2.5 cm long, terete to slightly flat, canaliculate, densely sericeous, trichomes similar to the branches; leaf blades chartaceous, discolorous,  $4.2-9.0 \times 1.5-4.6$  cm, plane, elliptic, widely elliptic to lanceolate, base rounded, apex acute, margins serrate, teeth  $1.5-3.0 \times 0.3-0.5$  mm, adaxial surface olive green to greyish-green, pubescent, abaxial cinereous, canescent, trichomes stellate, sessile; venation actinodromous, 7-9 pairs of secondary veins and 2 basal veins. Inflorescence cymose, axillary along the branches, short pedunculate, few-flowered; bracts  $5-6 \times 1.5-2.0$  mm, apex 2-3-dentate; peduncle 0.4-1.4 cm long, sericeous. Flowers distylous, sessile or short-pedicellate, arranged in pairs; bracteoles 4, 6.0–6.5  $\times$  1.0–1.5 mm, distinct, elliptic to narrowly elliptic, apex acute, entire to 2-3-fid, adaxial surface sericeous, trichomes stellate, abaxial surface with a layer of small and dense, canescent stellate trichomes and a layer of big and sparse, pilose stellate trichomes, sessile; veins 2-3, conspicuous. Calyx 5-merous, gamosepalous,  $4.5-5.5 \times 2.5-3.5$  mm, campanulate, 10-ribbed, pubescent externally, trichomes stellate and 2-fid, sessile, glabrous internally, internally pubescent on the lobes, simple trichomes, lobes  $2.8-3.0 \times 1.1-1.2$  mm, apex acute; 4-6 pairs of secondary veins; nectary ca 0.4 mm long, on the base of the internal surface. Corolla 5-merous, dialypetalous, pallid yellow, petals adnate to the staminal tube for ca 0.8 mm of the length,  $6.0-7.5 \times 2.0-2.2$  mm, spatulate, adaxial surface pilose, trichomes simple, abaxial surface glabrous, apex rounded to slightly emarginate, eciliate. Stamens 5, partially connate into a staminal tube, papillate apically, dithecal, thecae parallel, dehiscence rimose. Carpel 1 and locule 1, ovary obovoid, sericeous, style 1, lateral, pubescent, trichomes stellate, sessile, stigma 1, penicillate, fan-plumose. Brevistylous form: stamens ca 6.5 mm long, staminal tube 2.0–2.5  $\times$  0.8–1.0 mm, free filaments 3.1-3.5 mm long, anthers 1.1-1.2 mm long, gynoecium 2.0-3.5 mm long, ovary ca  $1.2 \times 0.8$  mm, sericeous apically, style 1.5 mm long, stigma ca  $1 \times 1$  mm. Longistylous form: stamens 4 mm long, staminal tube  $3.2 \times 1.2$  mm, free filaments absent, anthers 1.1–1.4 mm long, gynoecium 6.2–6.5 mm long, ovary  $0.5-1.1 \times 0.9$ mm, sericeous, style 4.2-4.5 mm long, stellate trichomes, stigma 1.1–1.2 × 1.1 mm, fan-plumose. Capsule 1, 2.0–2.5  $\times$  1.2–1.8 mm, obpyramidal, chartaceous at the apex, membranous below, apex truncate, sericeous, trichomes stellate, restricted to the apex, dehiscence loculicidal from the base to the apex and through it; seed 1,  $1.3-1.8 \times 0.8-$ 1.2 mm, obovoid, brown, glabrous, apex rounded.

**Distribution and habitat.** *Waltheria marielleae* is endemic to Brazil and known only from the states of Alagoas and Pernambuco (Fig. 4). This species is associated to the Atlantic Forest domain, found in seasonal semideciduous forests, as well as in the Caatinga (IBGE 2012), at elevations 300–700 m a.s.l.

**Phenology.** The species was collected with flowers from June to October and from December to January, and with fruits from June to October and in December.

**Etymology.** The specific epithet honours Marielle Franco, a sociologist and former Brazilian councilwoman who vehemently fought for social causes, including LGBTQI+ causes, a community that the first author is proud to be part of. Marielle Franco was cowardly murdered along with her driver Anderson Pedro Gomes in 2018, but her name remains alive in the memory of those who share her ideals.

**IUCN conservation assessment.** According to IUCN (2019) criteria and based on its Area of Occurrence (AOO of 28 km<sup>2</sup>) and Extent of Occurrence (EOO of 10,797 km<sup>2</sup>), *Waltheria marielleae* is assessed as Endangered (EN, B2ab (ii, iii)). The Atlantic Forest domain in the state of Pernambuco, where most of the populations were found, is highly fragmented with isolated and irregularly shaped patches, which can lead to population isolation (Trindade et al. 2008) and a decrease in the persistence of many species (Scarano and Ceotto 2015). A few populations are within protected areas such as Reserva Municipal de

	Waltheria ackermanniana	W. indica	W. marielleae	W. rotundifolia
Branch indumentum	Scabrous or strigose	Pilose or pubescent	Sericeous	Pubescent
Trichome type on branches	Stellate and sessile glandular	Stellate, associated or not with stalked-glandular	Stellate	Stellate
Leaf blade colour	Concolorous	Concolorous	Discolorous	Concolorous
No. of flowers per inflorescence	Few- or many-flowered (7–35)	Many-flowered (80-100)	Few-flowered (8-12)	Many-flowered (30–35)
Bracteole shape	Linear to elliptic	Elliptic	Narrowly elliptic to elliptic	Linear to lanceolate
Floral polymorphism type	Distylous	Homostylous	Distylous	Distylous
Calyx lobe shape	Long-acuminate	Long-acuminate	Acute	Acute
Petal apex indumentum	Ciliate	Ciliate	Eciliate	Ciliate
Capsule apex indumentum	Sericeous	Pilose or sericeous	Sericeous	Tomentose
Capsule dehiscence	Partial	Partial	Partial	Total

Table 1. Morphological comparison between Waltheria marielleae and related species.

Bonito (Pernambuco) and Reserva Biológica de Pedra Talhada (Alagoas), but others were recorded alongside roads and highways. In the Caatinga, *Waltheria marielleae* is not found in any protected area.

Additional material examined (paratypes). BRAZIL -Alagoas • Quebrangulo, Pedra Talhada; 6 Jan. 1986; fl.; Lyra-Lemos & Esteves 1140; HUEFS [000132912], MAC • Quebrangulo, Reserva Biológica de Pedra Talhada; 11 Jun. 2011; fl.; Lyra-Lemos et al. 13296; MAC • Quebrangulo; 23 Oct. 2011; fl.; Santos & França 21; MAC • Quebrangulo; 1 Aug. 2014; fl.; Correio et al. 134; MAC • Quebrangulo; 16 Dec. 2014; fl.; Nusbaumer 4169; JPB, MAC, NY [3474771] • São Sebastião, Povoado Sucupira; 1 Dec. 2003; fl.; Bayma s.n.; MAC [19983]. - Pernambuco • Bonito; 17 Sep. 1997; fl.; Santos 49; PEUFR • Bonito, Parque da Reserva Municipal; 17 Sep. 1997; fl.; Espíndola 08; PEUFR • Bonito, Reserva Municipal de Bonito; 13 Jun. 2018; fl.; Coutinho et al. 376; BHCB, CEN, EAC, HCDAL, JPB, MBM, UFP, RB, SPF • Bonito, Fazenda Tudo Muito; 13 Jun. 2018; fl.; Coutinho et al. 382; UFP; Bonito, Reserva Municipal de Bonito; 12 Sep. 1995; fl.; Melo et al. 245; PEUFR • Garanhuns, Fazenda Serra Branca; 09°10'38"S 40°58'20"W; 23 Oct. 2010; fl.; Oliveira et al. 5128; IPA • s.loc., margem da estrada entre Baraúna e Jurema; 26 Jul. 1966; fl.; Andrade-Lima 66-4712; IPA • s.loc., estrada para Petrolina; 5 Sep. 1986; fl.; Freitas 26; PEUFR.

#### Morphological affinities

*Waltheria marielleae* is classified in *W.* sect. *Waltheria* according to Schumann (1886) due to the capsules with loculicidal dehiscence and glabrous seeds. In addition, the narrowed stipules (narrowly triangular) and stellate trichomes on the branches can be used to categorize the

new species within this section. According to the informal classification proposed by Saunders (1995, unpubl.), *W. marielleae* is placed in *W.* subg. *Waltheria* sect. *Waltheria* by the capsule with chartaceous and truncate apex and apical dehiscence. *Waltheria marielleae* can be easily confused with *W. indica*, especially in herbaria specimens, due to the morphological characters indicated in the diagnosis and presented in Table 1.

*Waltheria marielleae* is phylogenetically related to *W. ackermanniana* and they share the subshrub habit, ciliate stipules, discolorous leaves, bracteoles longer than the calyx, distylous flowers, spatulate petals, and capsule with chartaceous, sericeous apex. *Waltheria marielleae*, however, has sericeous branches with only stellate trichomes (vs strigose or pilose branches with stellate and glandular trichomes in *W. ackermanniana*), adaxially pubescent leaves (vs scabrous), 6–6.5 mm long, elliptic to narrowly elliptic bracteoles (vs 8.5–11 mm long, linear), 2.8–3.0 mm long, acute calyx lobes (vs 3.8–4.0 mm long, acuminate), pallid yellow corolla (vs golden yellow or reddish-yellow), and eciliate petal apex (vs ciliate).

Waltheria marielleae also resembles W. rotundifolia by the greyish leaves, axillary and sessile to short pedunculate inflorescences, distylous flowers, calyx with acute lobe apex, and fan-plumose stigmas, but differs by the narrowly triangular stipules (vs linear), long-petiolate leaves (vs short-petiolate), discolorous leaf blades (vs concolorous), narrowly elliptic to elliptic bracteoles longer than the calyx, without glandular trichomes (vs linear to lanceolate, shorter than the calyx and with glandular trichomes), pallid yellow corolla (vs golden yellow), eciliate petal apex (vs ciliate), calyx 2.5–3.5 mm wide (vs 1.5–1.8 mm), capsule with sericeous apex and partial dehiscence (vs tomentose and total dehiscence). Regarding its geographic distribution, *W. marielleae* was collected together with *W. indica* in Bonito (state of Pernambuco), but the latter is a pantropical species, occurring in all regions and phytogeographical domains in Brazil. *Waltheria ackermanniana* was previously recorded in the south-eastern (states of Minas Gerais and

Rio de Janeiro) and north-eastern (states of Bahia and Pernambuco) regions (Coutinho and Alves 2020), being now also recorded in the state of Alagoas (*A.P. Fontana & P.M.G. Caxias 9836* (HPISF)). This species inhabits areas of Cerrado, Caatinga, and Atlantic Forest biomes. In the states of Alagoas and Pernambuco, where *W. marielleae* 



**Figure 2.** *Waltheria marielleae* (A–F), *W. ackermanniana* (G), *W. indica* (H), and *W. rotundifolia* (I). **A.** One population. **B.** General aspect showing greyish leaf indumentum. **C.** Detail of a flowering branch showing leaves and inflorescences arrangement. **D.** Stipule and inflorescence detail. **E.** Longistylous flowers showing the fan-plumose stigmas exserted. **F.** Brevistylous flowers showing stamens exserted. **G.** Detail of one inflorescence with a brevistylous flower. **H.** Inflorescence and detail of the homostylous flower. **I.** Inflorescence with brevistylous flowers.

was recorded, *W. ackermanniana* occurs ca 180 km and 200 km from the municipalities of Quebrangulo and São Sebastião, respectively, where the new species was collected.

In Brazil, *W. rotundifolia* occurs only in the northeastern region, in all states. Saunders and Dorr (2022) point out the occurrence of this species in the states of Minas Gerais and Rio de Janeiro, however, after an intense study of the botanical collections in Brazil and abroad, no specimen was found so that this data could not be validated. This species primarily inhabits caatinga vegetation, but in the state of Rio Grande do Norte, it was observed growing in restinga vegetation (savannalike formation with beach sands deposited during the Quaternary, in areas above sea level that support scattered clumps of small trees, shrubs, herbs, and grasses (Thomas and Barbosa 2008)), an environment where *W. marielleae* has not been recorded.



**Figure 3.** *Waltheria marielleae.* **A.** Flowering branch with detail of the trichomes. **B.** Bracteoles. **C.** Brevistylous flower. **D.** Longistylous flower. **E.** Calyx showing internal surface. **F.** Calyx lobe with trichomes removed showing veins. **G.** Petal (adaxial surface). **H.** Stamens and part of the gynoecium of a longistylous flower. **I.** Stamens and stigma of a brevistylous flower. **J.** Gynoecium of a brevistylous flower. **K.** Capsule. **L.** Seed. A, B, D–H from *Coutinho et al. 375* (UFP, holotype); C, I–L from *Coutinho et al. 376* (UFP, paratype). Drawn by Regina Carvalho.

### Key to the Waltheria species occurring in Alagoas and Pernambuco, Brazil

1.	Foliaceous stipules, rounded base; capsules with operculate dehiscence; verrucose seeds (W. sect. Stegowaltheria)
-	Scarious stipules, truncate base; capsule with loculicidal dehiscence; smooth to slightly crenulate seeds (W. sect. Waltheria) 3
2.	Prostrate herbs; distylous flowers
-	Erect herbs; homostylous flowers
3.	Sticky branches and leaves; leaf blade with acuminate apex
-	Non-sticky branches and leaves; leaf blade with acute, rounded, truncate, or emarginate apex
4.	Terminal inflorescences
-	Axillary inflorescences
5.	Xylopodium present; filiform stipules; capsule with total dehiscence
-	Xylopodium absent; narrowly lanceolate stipules; capsule with partial dehiscence
6.	Homostylous flowers
-	Distylous flowers
7.	Strigose or scabrous branches, stellate and glandular sessile trichomes
-	Tomentose, sericeous, pilose, or pubescent branches, stellate trichomes, with or without glandular stalked trichomes
8.	Branches with glandular stalked trichomes; calyx with acuminate apex lobe; elongate-plumose stigmas
-	Branches only with stellate trichomes or mixed with glandular sessile trichomes; calyx with acute apex lobe; fan-plumose stigmas9
9.	Discolorous leaf blades; elliptic to narrowly elliptic bracteoles with stellate trichomes; capsules with sericeous apex <i>W. marielleae</i>
-	Concolorous leaf blades; linear to lanceolate bracteoles with stellate and glandular trichomes; capsules with tomentose apex
	W. rotundifolia



**Figure 4.** Geographic distribution map of *Waltheria marielleae* and new records of *W. ackermanniana* and *W. rotundifolia*. AL = Alagoas, PE = Pernambuco.

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# SUPPLEMENTARY FILES

## Supplementary file 1

Malvaceae species with voucher information and DNA regions used for the phylogenetic reconstruction, including GenBank accession numbers, also for the outgroup. Link: https://doi.org/10.5091/plecevo.94921.suppl1

#### Supplementary file 2

Statistics of the markers used in the analyses. For each marker, the number of accessions analysed, the number of aligned characters (bp), the percentage of variable and conserved characters, and the substitution model are presented. Link: https://doi.org/10.5091/plecevo.94921.suppl2