

# *Schütziana*

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Cover picture: *Gymnocalycium kuehhasii* SPE 131-57-13, TS 169, 6 km north of Villa de Maria, province Córdoba, Argentina, 480 m. a. s. l. (photograph: Reiner Sperling).

## Editorial

### Dear *Gymnocalycium* friends

Mario Wick



The hilly regions of northern Córdoba as well as the southern part of the neighbouring province Santiago del Estero were not really popular with travellers in *Gymnocalycium* matters about 10 to 15 years ago. At that time there were only inexact maps of that region and, in addition, there was no systematic approach to the genus. Our team “Gymnos” therefore decided to have a closer look at that area.

Since then various collectors have been travelling in the area and collected an abundance of information about the plants growing there. Especially in recent years this yielded descriptions of beautiful new species such as *Gymnocalycium alenae* Kulhánek (2017), *Gymnocalycium campestre* Řepka (2015) and *Gymnocalycium affine* Řepka (2010).

However, it is not easy to establish order among the *Gymnocalycia* in northern Córdoba. Almost all occurring species show morphological gradations between neighbouring species in the peripheral regions of their distribution area or imitate their features and appearance respectively. This also applies to the fringe populations of *Gymnocalycium kuehhasii*, which so far could not be attributed unambiguously. But now, by analysis of ploidy levels with the help of flow cytometry method, it will certainly be possible to distinguish them from similar populations of sympatric species with different ploidy levels.

In the third part of his series about the *Gymnocalycium berchtii* species group Tomáš Kulhánek presents *Gymnocalycium morroense*. For his contribution to this edition he used the flow cytometry method to establish ploidy levels of the various populations analysed. Just like in northern Córdoba there seems to exist another hot spot of species development in the province San Luis. All populations reviewed had a polyploid set of chromosomes.

Enjoy yourselves reading!

We would like to express our warmest thanks to Mrs Iris Blanz (Fernitz, Austria), to Mr Brian Bates (Bolivia) and to Mr Graham Charles (United Kingdom), who support us with the translation into English, to Mrs Larisa Zaitseva for the translation into Russian (Tscheljabinsk, Russia), to Mr Takashi Shimada (Japan) for the translation into Japanese and to Mr Daniel Schweich (France), who has mirrored our publications under <http://www.cactuspro.com/biblio/>.

## ***Gymnocalycium morroense* and comparison with related species**

**Tomáš Kulhánek**

Tylova 673, CZ-67201 Moravský Krumlov (Czech Republic)

E-mail: [tomas.kulhanek@schuetziana.org](mailto:tomas.kulhanek@schuetziana.org)



### **ABSTRACT**

The third part extends the series about the *G. berchtii* species group. It deals with *G. morroense*. Type locality, occurrence area and characteristics of the species are demonstrated. The main features distinguishing *G. morroense* from other related taxa which may enter its area of occurrence (namely *G. sutterianum* s. l., *G. nataliae* s. l. and *G. poeschlii*) are noted.

**KEYWORDS:** *Cactaceae*, *Gymnocalycium*, *berchtii*, *morroense*, *nataliae*, *poeschlii*, *sutterianum*.

The name corresponding with *G. morroense* used to be first mentioned as nomen prov. for plants occurring in the area of the Sierra del Morro and the Sierra del Rosario (Tiporco Rosario), San Luis province in the Czech bulletin of Gymnofil 2007, part 2. (Kulhánek 2007). In this short article, the author puts forward his first ideas about possible areas where already described taxa of the subgenus *Gymnocalycium* occur, **based on their phenotypical characters, instead of those with diploid sets of chromosomes** (Fig.1). Later, after further field studies in 2009 and 2010, the epithet *morroense* was used for those plants and *G. morroense* was validly published (Kulhánek et al. 2010).

***Gymnocalycium morroense*** Kulhánek, Řepka et J. Proch.

[*Gymnocalycium* 23 (3) 2010: 947-954]

**Typus (holotypus):** Argentina, prov. San Luis, Sierra del Morro, El Guanaco del Morro, ad marginem fruteti secus viam publicam 0,7 km or.-sept.-or. a jugo montis Cerro Guanaco, 960 m s. m., leg. R. Řepka ut no. RER 432, die 20.01.2010, CORD (corpus cum fructus in liquoro alcoholico); **isotypus** in herbario WU (corpus in liquoro alcoholico).

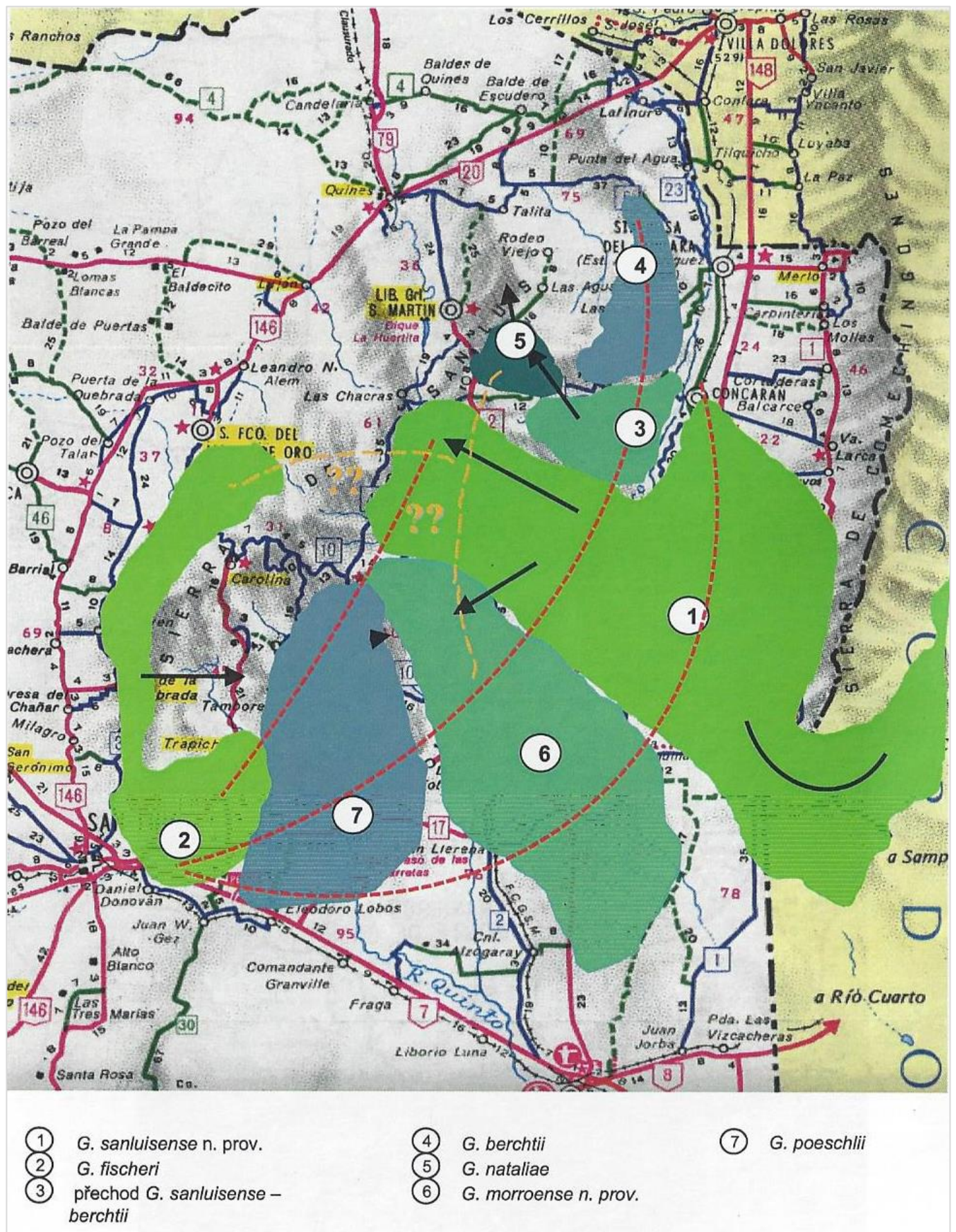


Fig. 1: First ideas about distribution of known species in prov. San Luis (Kulhánek 2007) at that time.



Fig. 2: Type plant and Sierra del Morro with Cerro Guanaco and Sierra Yulto, winter view in southern direction.

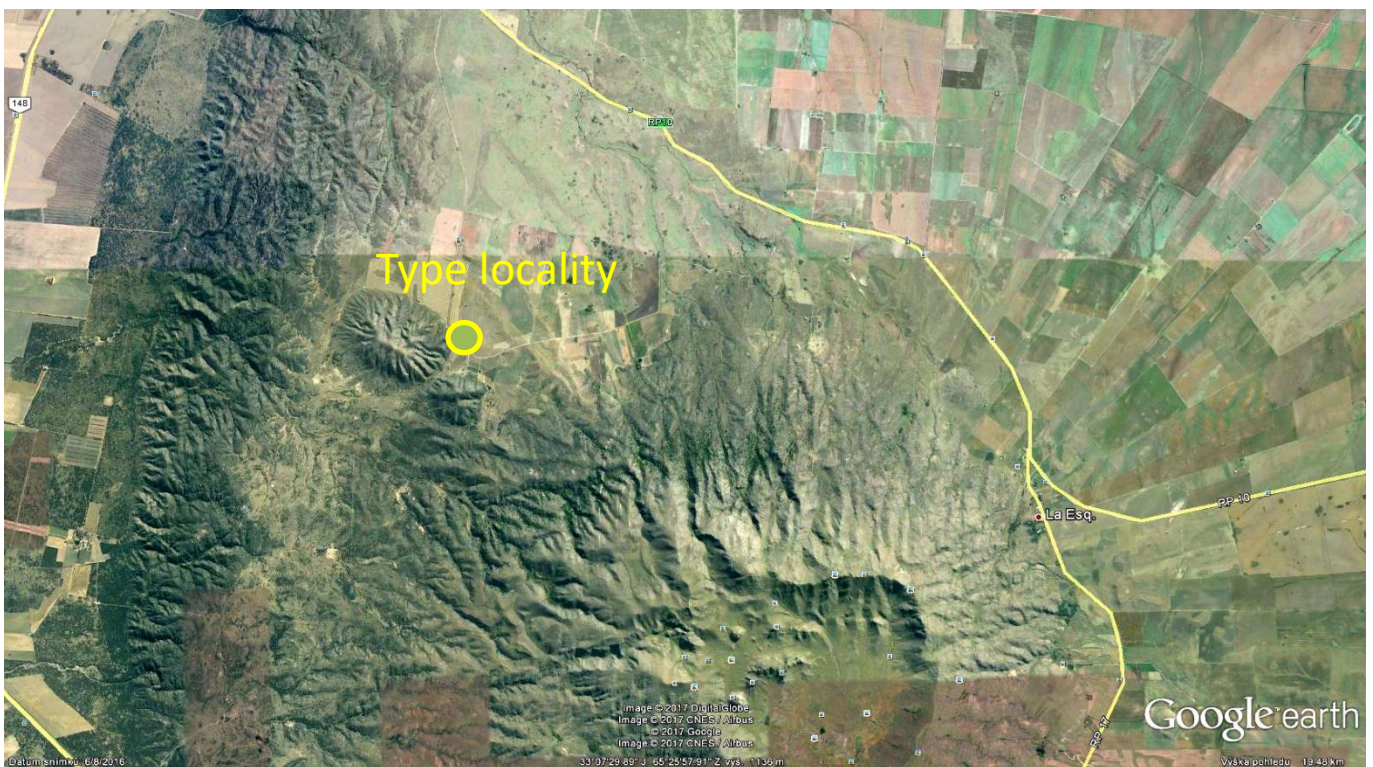


Fig. 3: Sierra del Morro and indicated place where the holotype and isotype were collected.

The holotype was collected and deposited by the second author of the first description in 2010. This habitat had already been visited by all authors in 2009. The population from which the type plant with fruit (fig. 2 and 4) was collected occurs on the north-eastern side of the Cerro Guanaco foothill at an altitude of 1060 m. a. s. l. instead of 960 m. a. s. l. as indicated in in the protologue by mistake. The plant deposited as isotype by the second author is illustrated in fig. 5.



Fig. 4: Plant with fruit collected and deposited as holotype with no. RER 432, CORD.



Fig. 5: Plant deposited as isotype in WU, **A** photographed as Tom 09-414 in 2009, **B** the same plant 2010, photographed and collected as RER 432 (ISO).



Fig. 6: Type locality of *G. morroense* at the base of the Cerro Guanaco del Morro.

**Diagnosis:** *Speciebus Gymnocalycio poeschlii, G. nataliae et G. berchtii affinis, sed corpore 50-80 lato cum epidermide griseo-viridi sine pellicula grisea, costis applanatis, in tubercula hemisphaerica cum gibba subangulata divisis, spinis 5-7, juventute gibbam circumdantibus, demum rectis, radiantibus, eburneis, 7-10 mm longis differt. Flos hermaphroditus, roseus usque pallide roseus, cum fauce intense rosea. Fructus obovatus usque clavate doliiformis, azureo-viride pruinosis.*

The **Description** of this species was made based on the variation of the plants studied in the type locality (fig. 7). In the publication of the description we also published pictures of this species from the other localities, but they do not influence the description (it also covers the considerable variation of plants in other populations corresponding to this species) (Kulhánek et al. 2010). Therefore, an emendation of this species is not necessary.

The detailed description of the species and a comparison table of characteristics with other possibly related taxa is given in the original article (Kulhánek et al. 2010).

## ETYMOLOGY

The epithet “morroense” is derived from the name ‘Sierra del Morro’.





Fig. 7: *G. morroense* at type locality, at the base of Cerro Guanaco del Morro, 1060 m. s. m., San Luis.

## ECOLOGY

What we consider as belonging to *G. morroense* populates very moderate slopes or flat parts of small hills. The plants are mostly submerged between crumbled stones in a shallow layer of soil or can grow in rock crevices filled with soil. The most frequented habitats are presented in fig. 8. Habitats are mostly degenerated pastures with *Acacia caven* as the dominant plant of the Chaco seco ecoregion. In these habitats, there are species-rich grassland phytocenoses (grassy steppes included *Stipa*, *Festuca*) with occasional shrubs with low habitation rate. **Romerillal** (fig. 6 and 8) is one of the most frequent biocoenosis in habitats of *G. morroense* where *Eupatorium buniifolium* and *Heterothalamus alienus* (*Asteraceae*) are very often dominant shrubs. This species tolerates shaded areas poorly, shaded plants show completely unusual features.

Accompanying species from the *Cactaceae* family growing syntopically can be *Gymnocalycium lukasikii*, *G. achirasense*, *Echinopsis aurea* and *Notocactus submammulosus*. In habitats at lower altitudes than those, mostly *G. borthii* var. *viridis* and rarely *Pterocactus spec.* grow sympatrically.

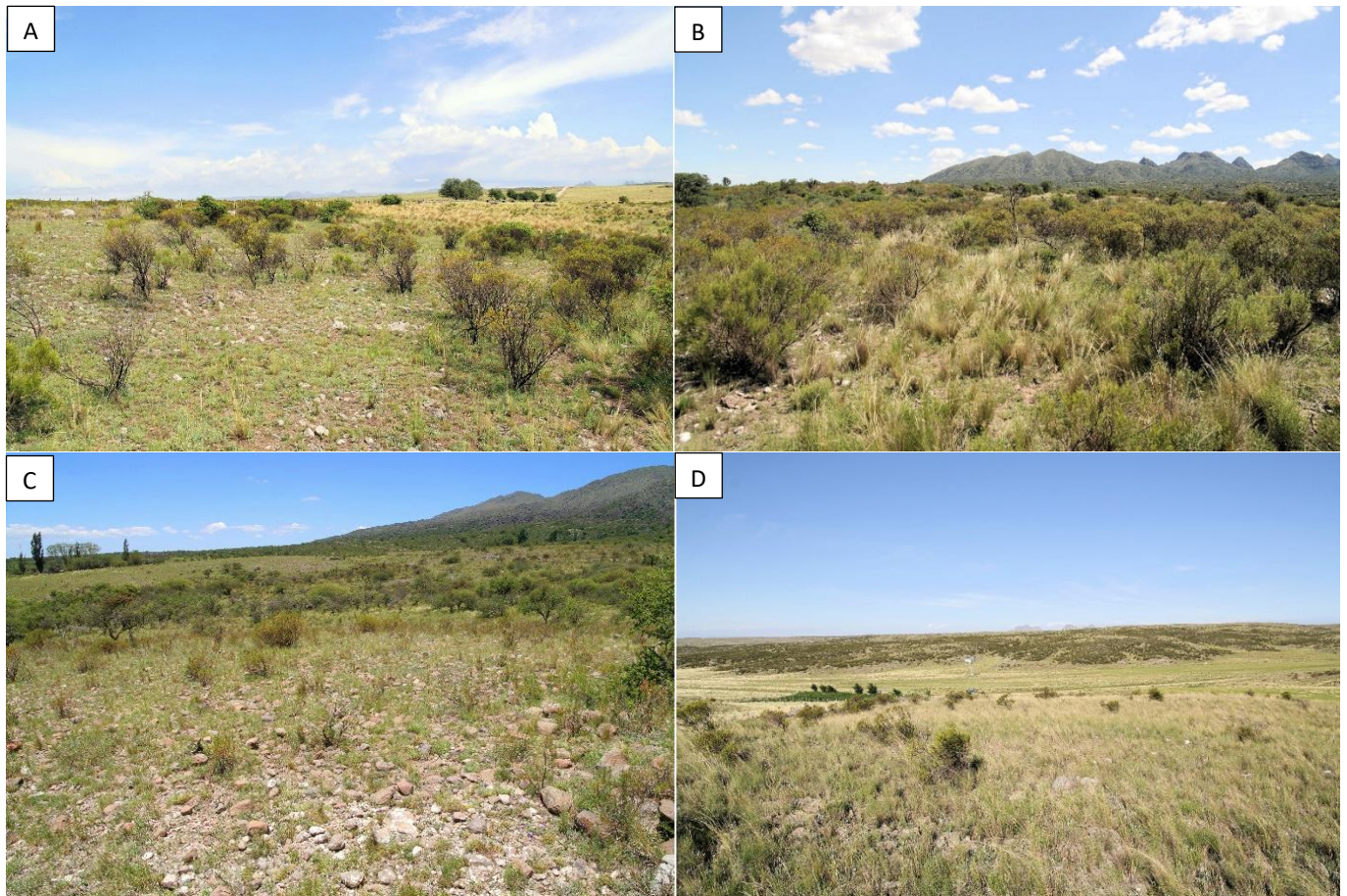


Fig. 8: Most frequent types of habitat where *G. morroense* occurs, **A** Romerillal N of Los Membrillos, **B** Romerillal S of Paso Grande, **C** Estepas near Cerro Guanaco, Sierra Del Morro, **D** habitat near Cruz Brillante on biotitic schists and gneisses.

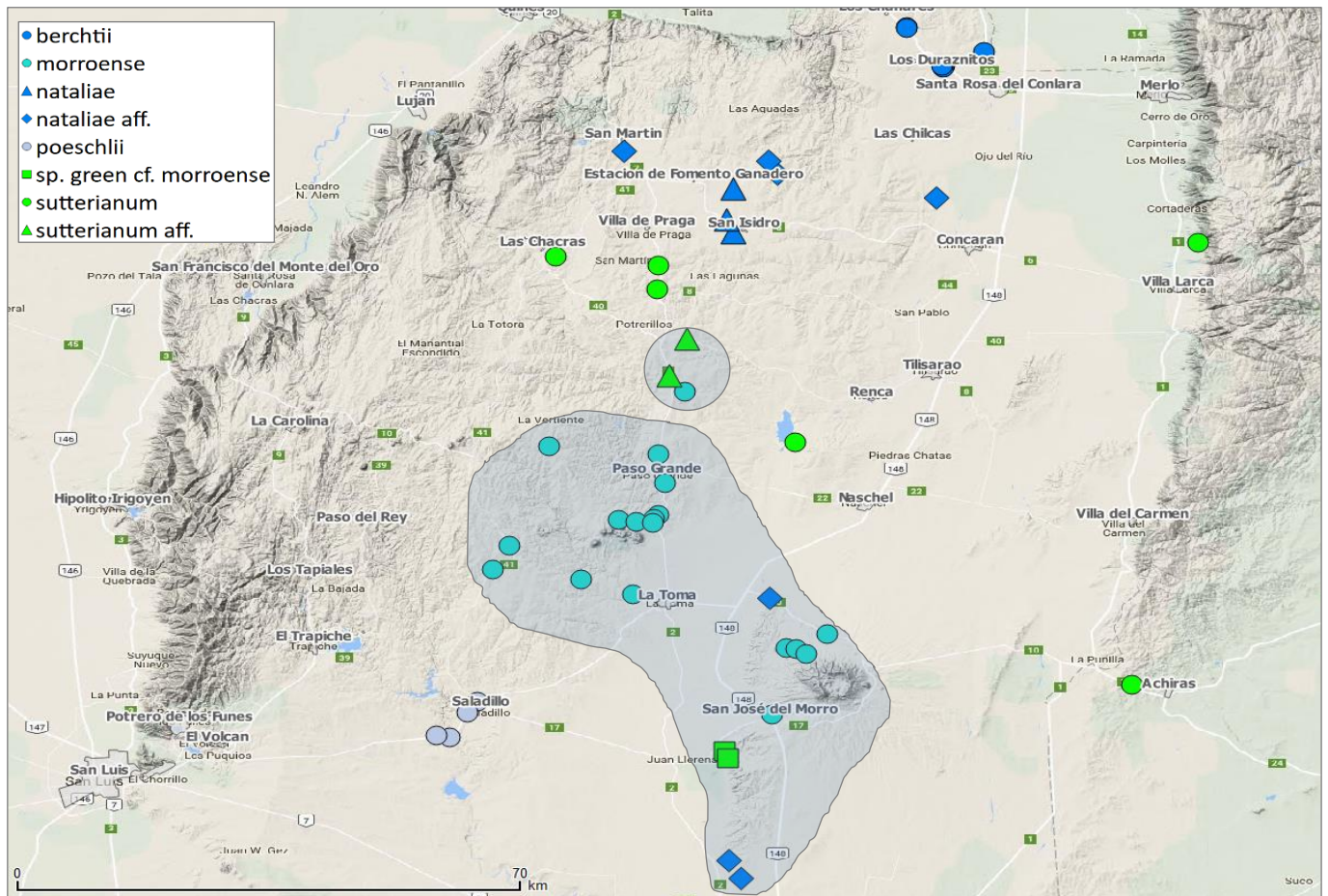


Fig. 9: Distribution map of *G. morroense* and other related taxa.

### AREA OF OCCURRENCE

The area where *G. morroense* occurs is not only around the Sierra del Morro but extends to the northern part of the Sierra del Tiporco Rosario where habitats of *G. morroense* have been studied between the villages Paso Grande and Los Vertientes (fig. 9). The northernmost occurrence was recorded close to R2 between Paso Grande and Los Potrerillos. The area of *G. morroense* extends between areas of other species or touches their borders, especially *G. nataliae*, *G. poeschlii* and *G. sutterianum* s. l. (incl. *G. sanluisense*). The morphologically different species *G. lukasikii* (diploid) has its own distribution area inside the area of *G. morroense* or may extend beyond it. *G. berchtii* is completely isolated in an area N and NW of Santa Rosa del Conlara. Different populations of *G. morroense* are illustrated in fig. 10-17.

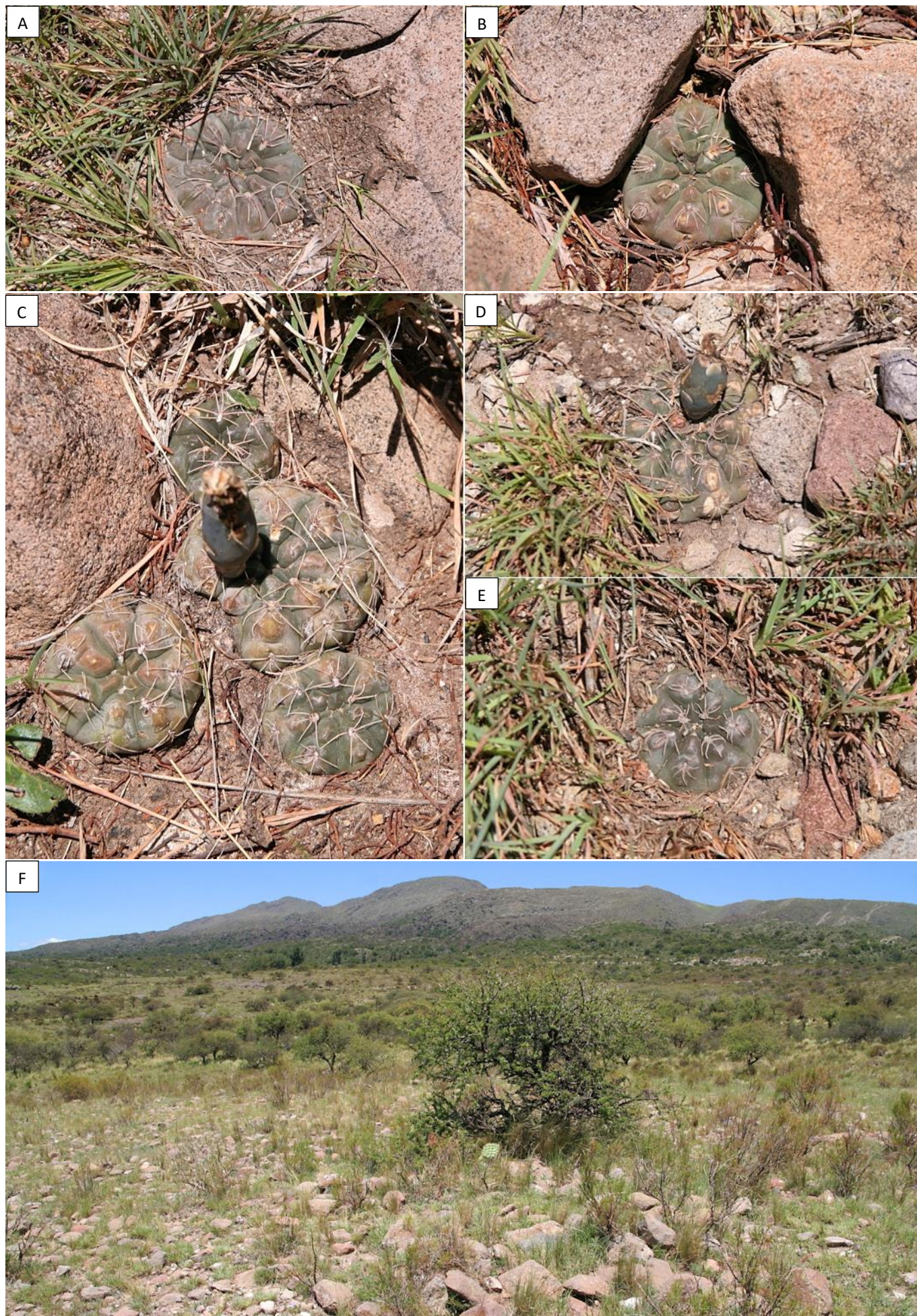


Fig. 10: Variability of *G. morroense* in habitat Tom 09-416.

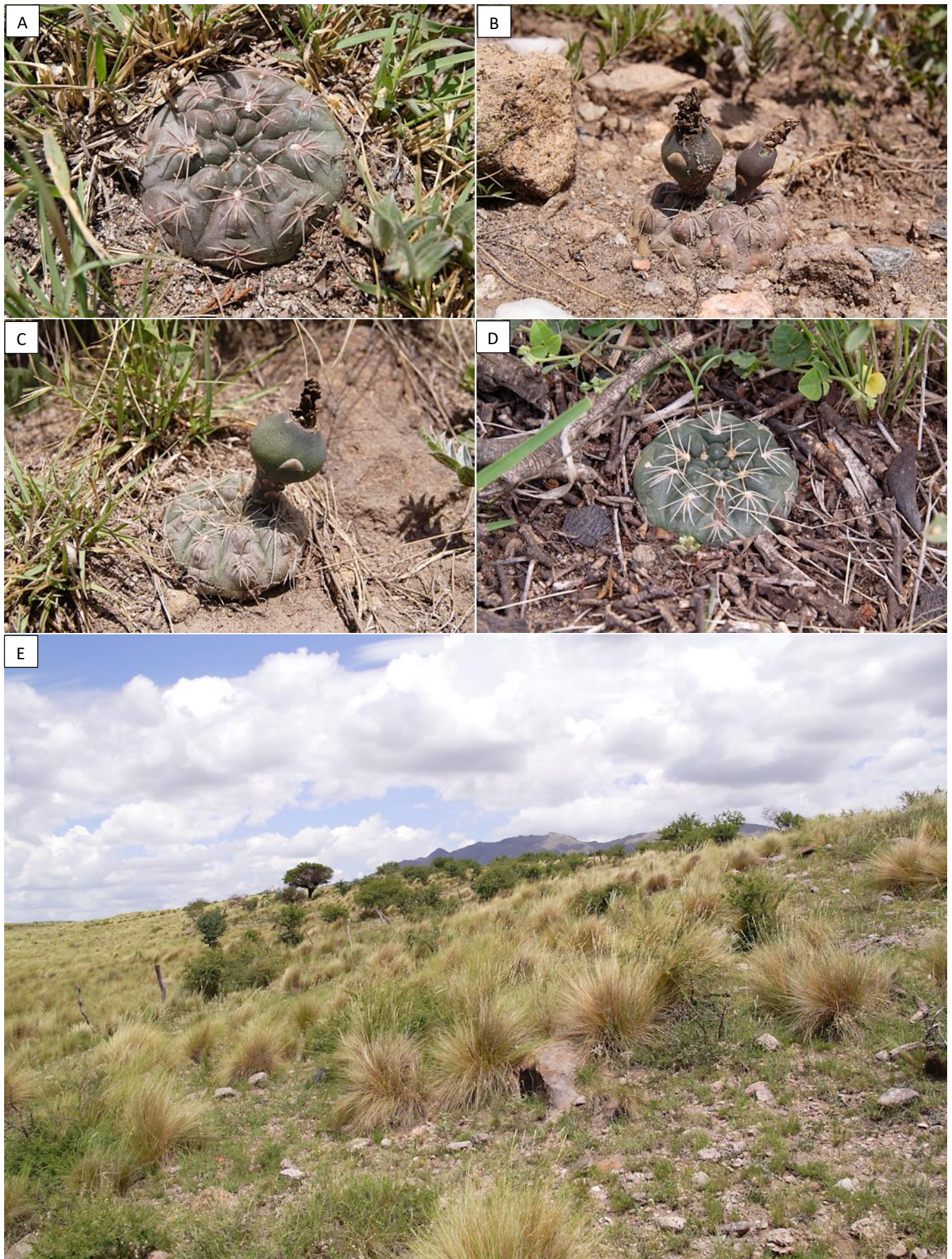


Fig. 11: Variability of *G. morroense* in habitat Tom 06-179.

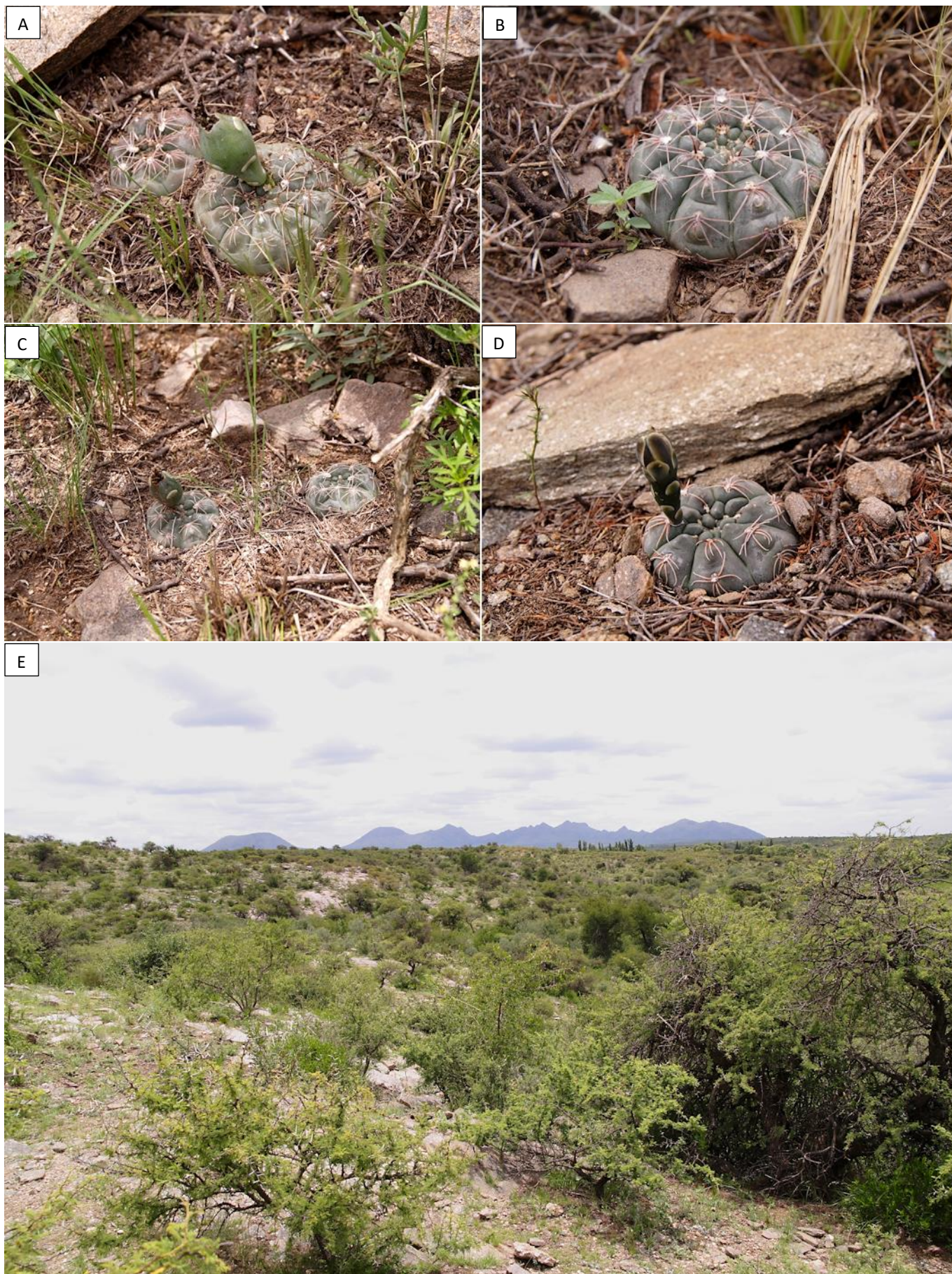


Fig. 12: Variability of *G. morroense* in habitat Tom 06-173.

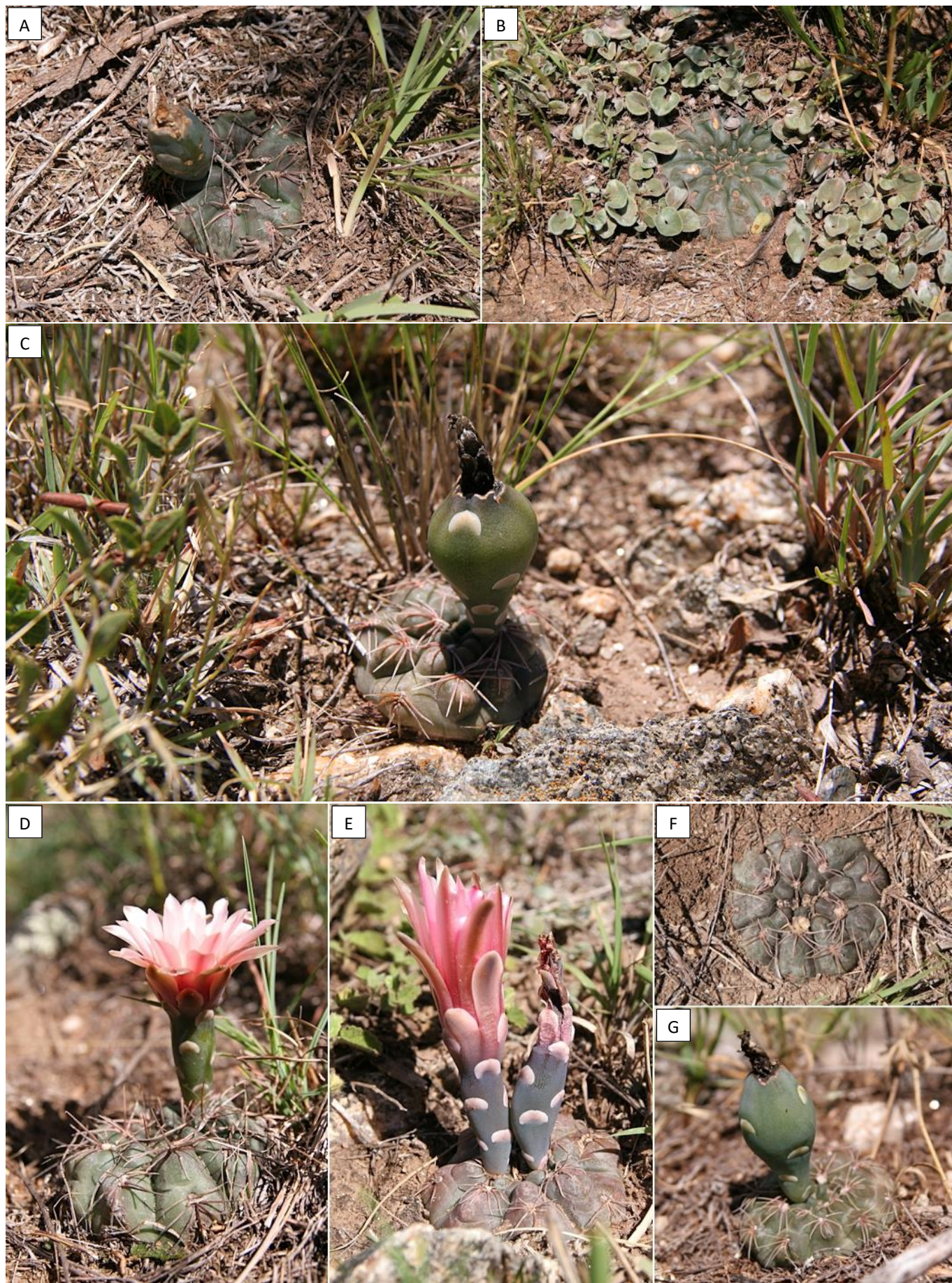


Fig. 13: Variability of *G. morroense* in habitat Tom 09-408 (A-C), Tom 09-409 (D-G).

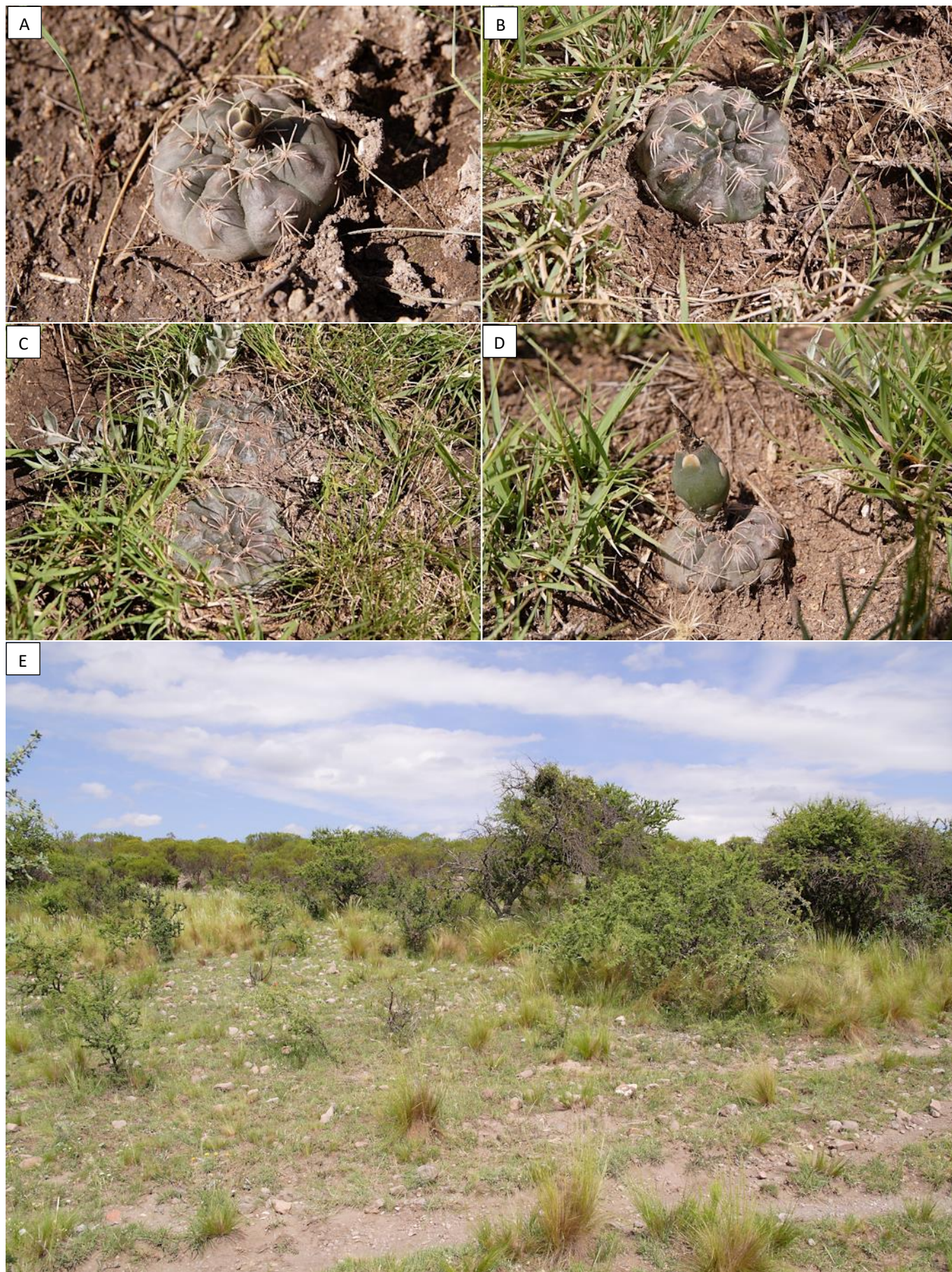


Fig. 14: Variability of *G. morroense* in habitat Tom 07-208.





Fig. 15: Variability of *G. morroense* in habitat Tom 09-411 (A-B), Tom 09-412 (C-F), Tom 07-198 (G).

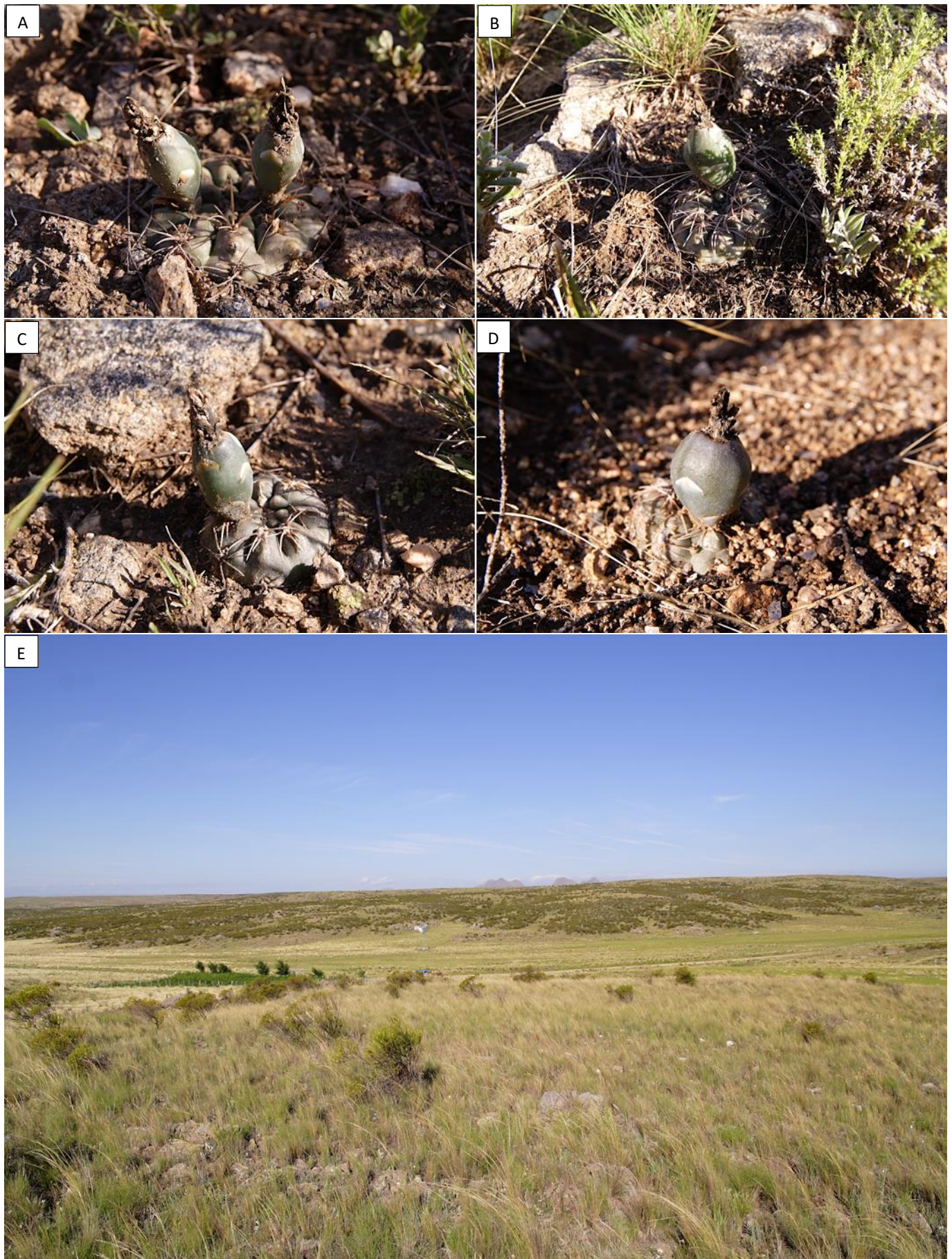


Fig. 16: Variability of *G. morroense* in habitat Tom 07-207.



Fig. 17: Variability of *G. morroense* in habitat Tom 07-211.

## The distinction of *G. morroense* from related and similar species

Like other species of the subgenus occurring in San Luis province, there is a considerable variation in body size, flowers and fruits, pericarp length and its colour, but most variation is in the arrangement and length of spines. For *G. morroense* there is a distinctive difference in the arrangement of spines between young and adult, respectively old plants. In some localities, *G. morroense* plants have longer spines, larger humps on the ribs and greener epidermis; they may be similar to *G. sutterianum* phenotype (*G. sanluisense*): however, the features of *G. morroense* remain with greyish pericarp and pinker flowers and also the shorter adjacent spines typically bent to the body in young plants. It concerns the northern and north-western parts of the *G. morroense* area (area south of Las Chacras, south of Potrerillos), but also the north-eastern parts (in direction Potrerillos to Naschel) where introgression of *G. sutterianum* (*G. sanluisense*) features can be assumed or has been studied. Such a population with more characteristics referring to *G. sutterianum* was indicated close to Potrerillos. This fact was then supported by flow cytometry (see annex 1) where all studied *G. sutterianum* (incl. *G. sanluisense*) in San Luis province are hexaploid ( $2n = 66$ ) and on the other side all *G. morroense* are tetraploid ( $2n = 44$ ). Significant difference in genome sizes was also recorded. This was measured for *G. morroense* between 8.2 and 8.6 pg (picogram) and for *G. sutterianum* close to 12 pg (Řepka et Koutecký unpubl.).

The similarity of *G. morroense* can also be demonstrated with *G. poeschlii*, which differs in 5 long spines, significantly spread from the body and early flowering dioecious flowers. On the southwest edge of the *G. morroense* area plants can be found that retain some of their features.

*G. nataliae* grows in the area north of *G. morroense* and differs in its smaller, often brownish coloured body with a metallic tinge and with thin, shorter spines, which are mostly spread in all directions. Flowers can be similar in both species, but the *G. morroense* flower seems to be more robust in contrast to the narrow flower of *G. nataliae*. The fruits are brownish green, fully mature purple or pink coloured. *G. morroense* contrasts in having a more greyish green body with more prominent bumps. In the southern distribution area on the edges of foothills belonging to the Sierra del Morro in the north and the Sierra Yulto in the south, *G. morroense* borders on populations with more characteristics corresponding with phenotypes of *G. nataliae* (fig. 22, compare fig. 22 with fig. 24 A-E). These populations are noticeable when combining the features of purple-brown epidermis and dark spines. The other features correspond to *G. morroense*, but with a significant contribution of other species - *G. nataliae* (see Schütziana 2(2011)1 p. 22-24). *G. nataliae* is also tetraploid ( $2n = 44$ ) with a very small difference in genome size compared to *G. morroense* (Řepka et Koutecký unpubl.). The difference between these two taxa (*G. morroense* and *G. nataliae*) can be distinguished only by morphological features as mentioned above. On the northern and north western edge of the *G. nataliae* occurrence, the area where the type of *G. nataliae* was collected, populations with morphological phenotypes related to *G. morroense* or to both species can be found (see Schütziana 2(2011)1 p.14-15, fig. 35, 36, 40).

The most important morphological features distinguishing *G. morroense* from all other relatives are the following: in young plants, adjacent spines are on the bumps of the ribs, the tips can be hooked and their colouring is ivory; in adults spines stand upright to straight, without a dark base. Flowers are bisexual, pericarp and then fruits are olive greyish green with more or less developed bloom (this variability has been recorded in all studied populations), fruit mostly obovate with or without developed peduncle. The morphological differences described can also be seen in

illustrated pictures of adult plants (fig. 18) and in comparison with juvenile plants of *G. sutterianum* (fig. 20), *G. morroense* (fig. 22) and *G. nataliae* (fig. 24). All taxa in fig. 26 are presented with their ploidy levels. An influence of diploid populations with morphological features of *G. borthii* or *G. lukasikii* subsp. *emillii* on populations of the *G. berchtii* species group (agg.) is questionable. However, some features of these diploid genotypes can be seen in habitats where taxa from both groups can grow syntopically or sympatrically. The features are mainly those three: brownish epidermis with metallic tinge, darker spines and fruits with brownish colouring and sometimes with a metallic tinge. These features can be seen in phenotypes of *G. nataliae* from the area of the type but also from its southern distribution area, see *G. nataliae* aff. (Kulhánek 2011) and *G. berchtii* populations. A fruit comparison of all relatives is illustrated in fig. 27.

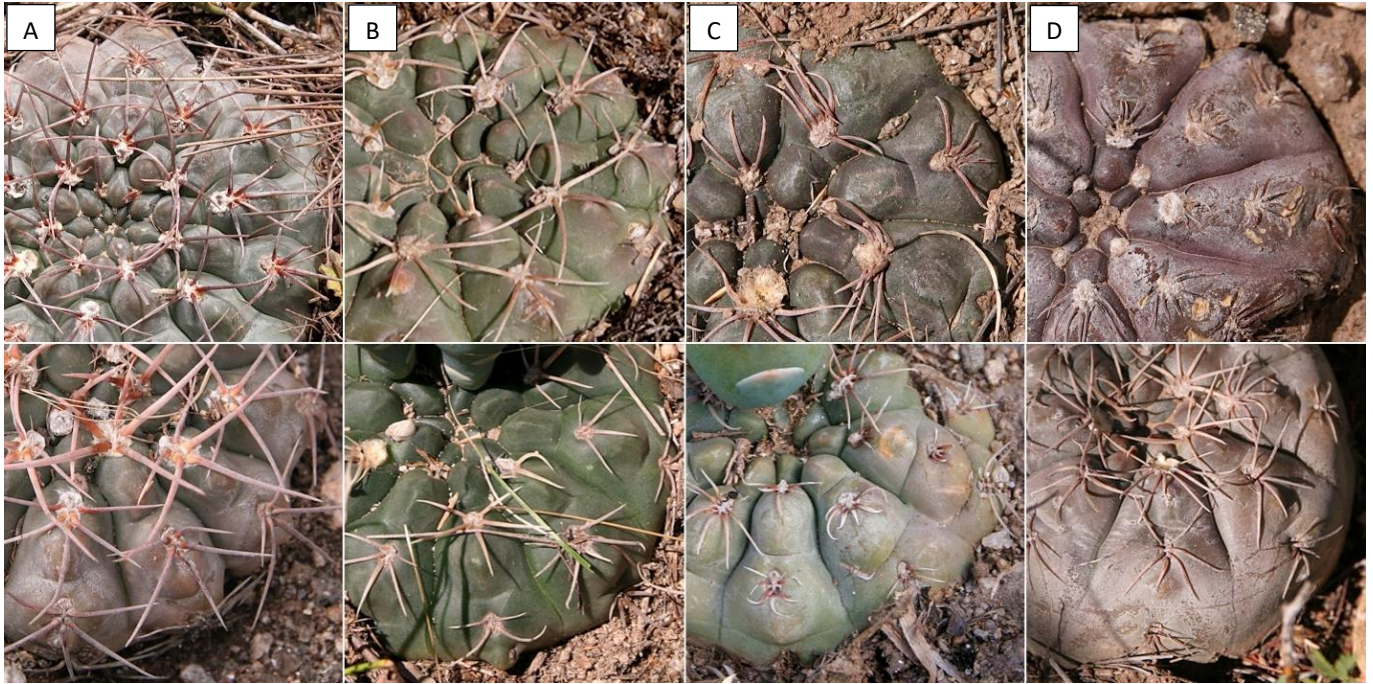


Fig. 18: Comparison of main morphological features of *G. morroense* with other related taxa; **A** *G. poeschlii*, **B** *G. sutterianum*, **C** *G. morroense* and **D** *G. nataliae*.

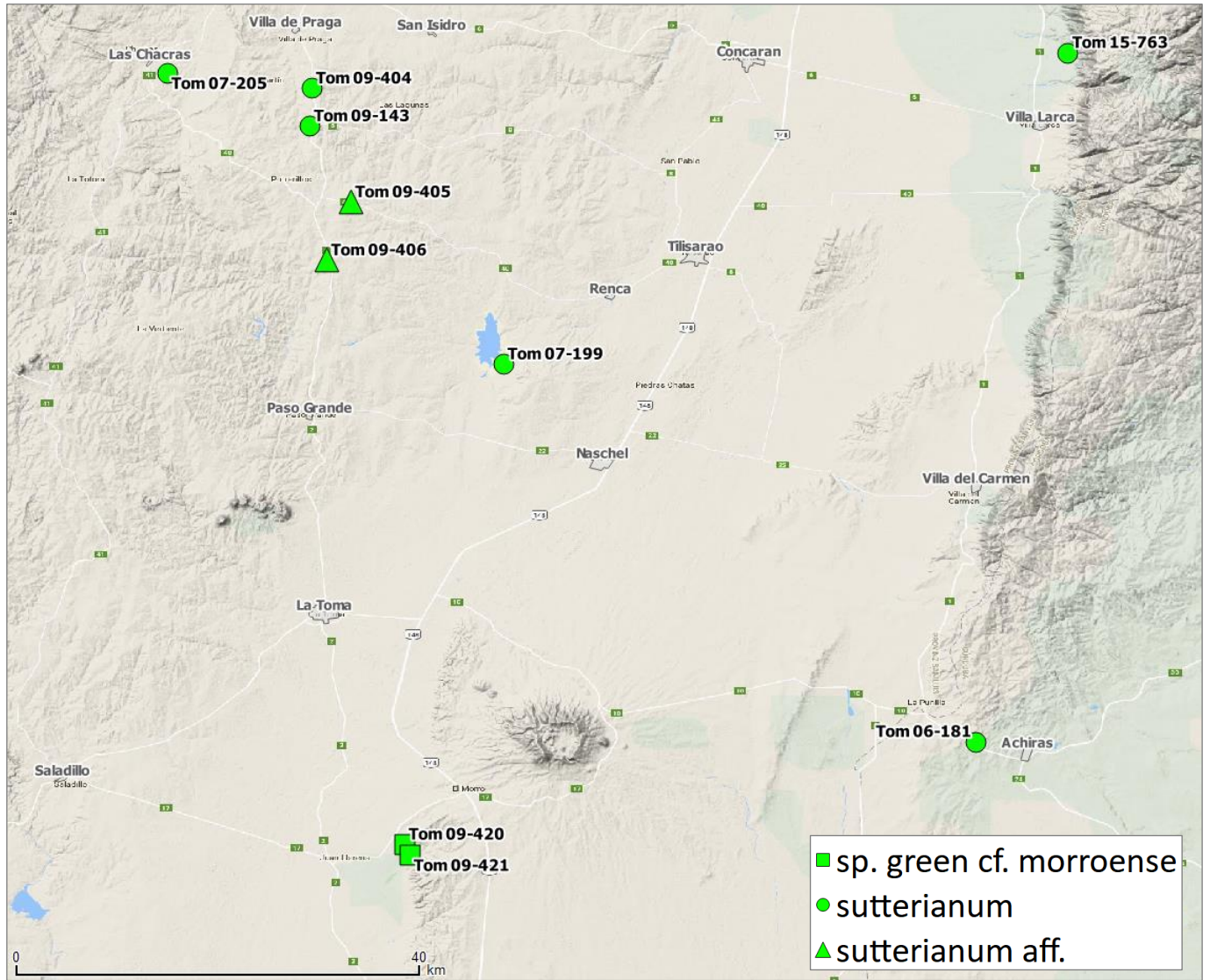


Fig. 19: Distribution map of *G. sutterianum*.

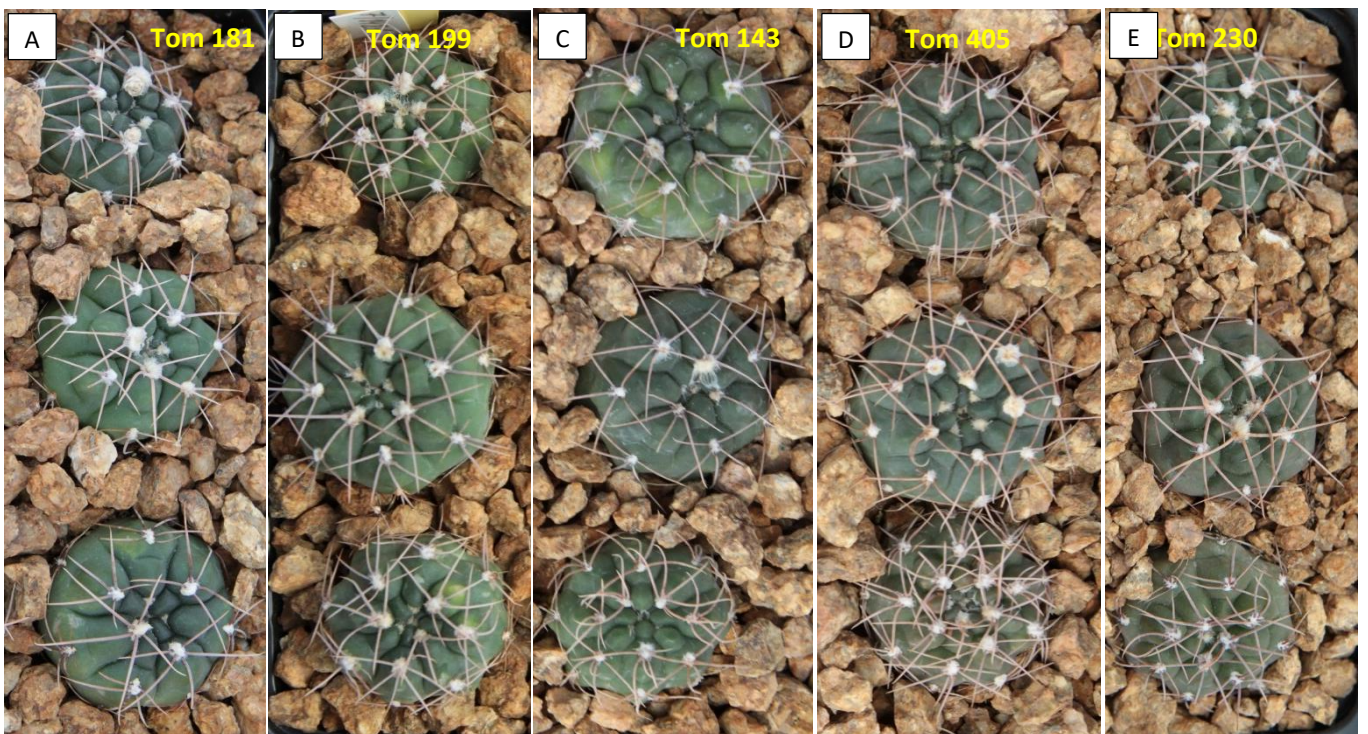


Fig. 20: *G. sutterianum*  $2n = 66$ ; **A** Tom 06-181, **B** Tom 07-199, **C** Tom 06-143, **D** Tom 09-405, **E** Tom 07-230.

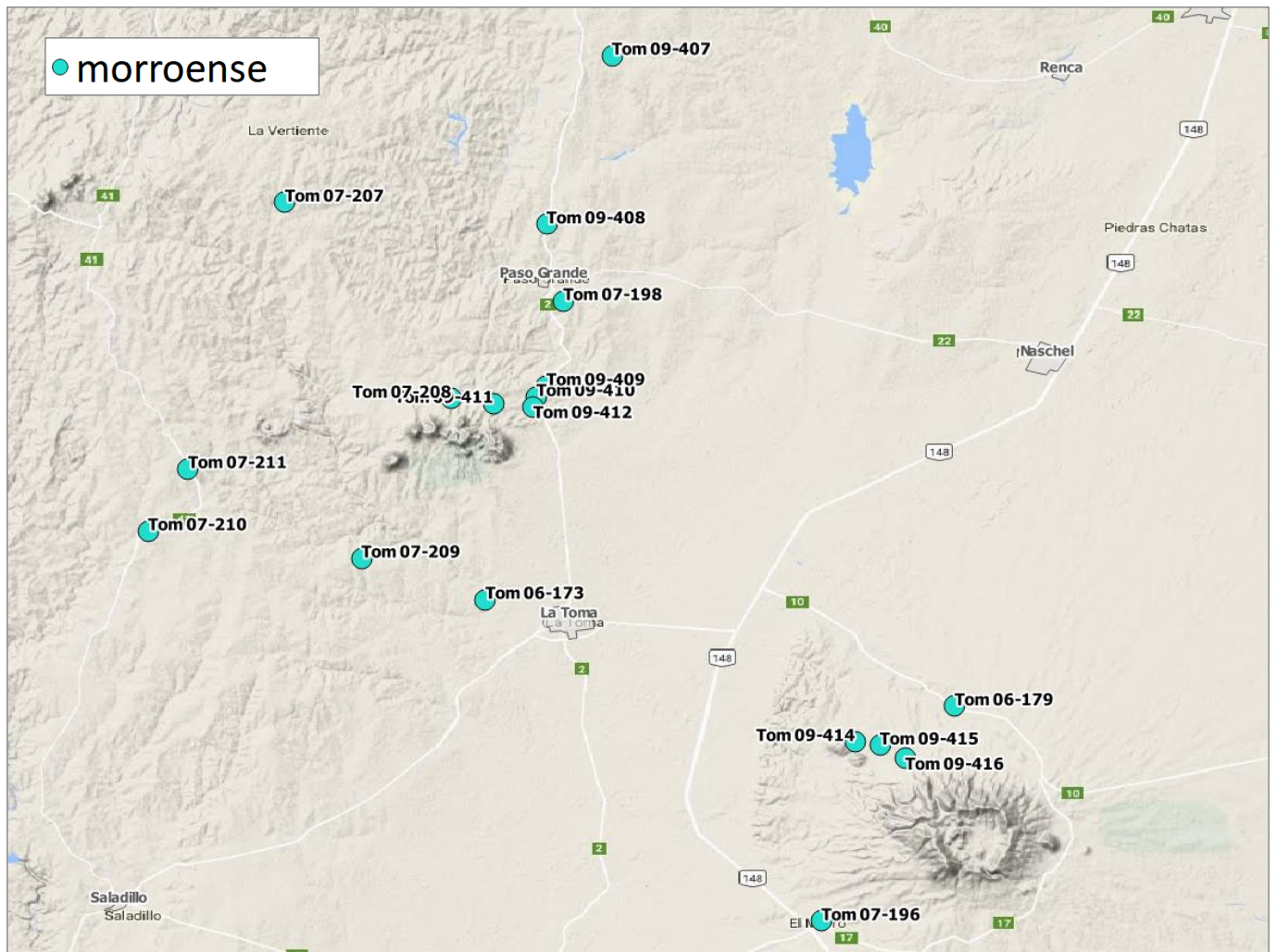


Fig. 21: Distribution map of *G. morroense*.

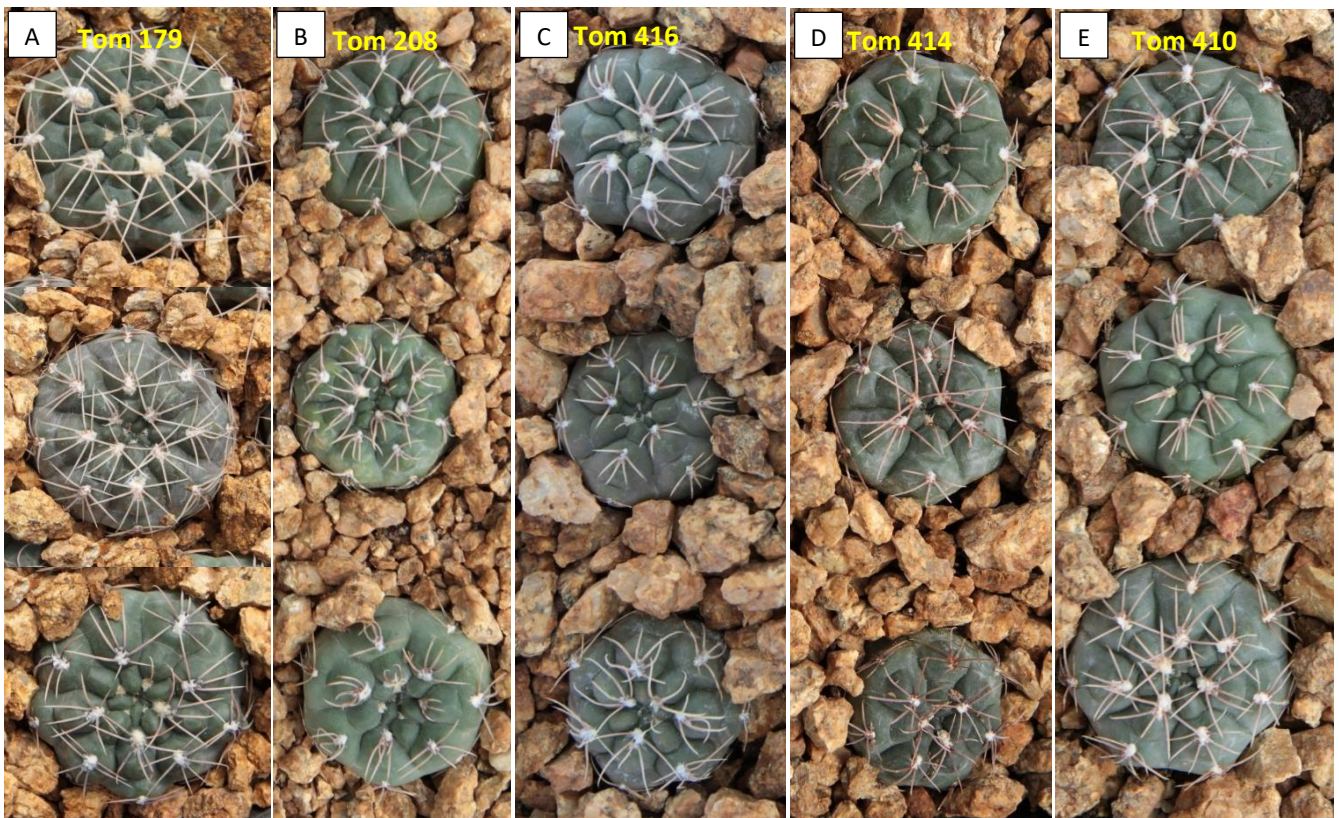


Fig. 22: *G. morroense*  $2n = 44$ ; A Tom 06-179, B Tom 07-208, C Tom 09-416, D Tom 09-414, E Tom 09-410.

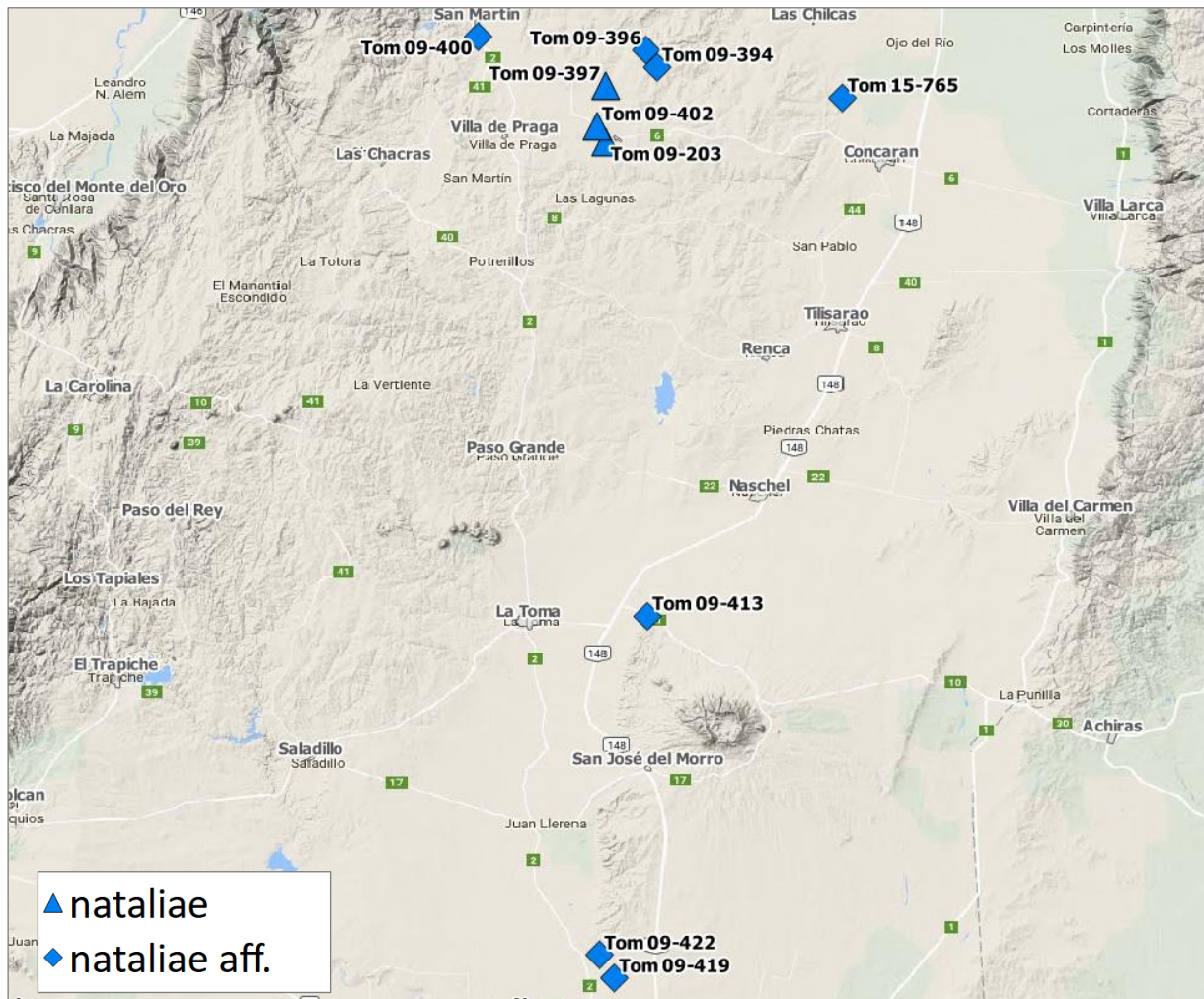


Fig. 23: Distribution map of *G. nataliae* and *G. nataliae* aff.

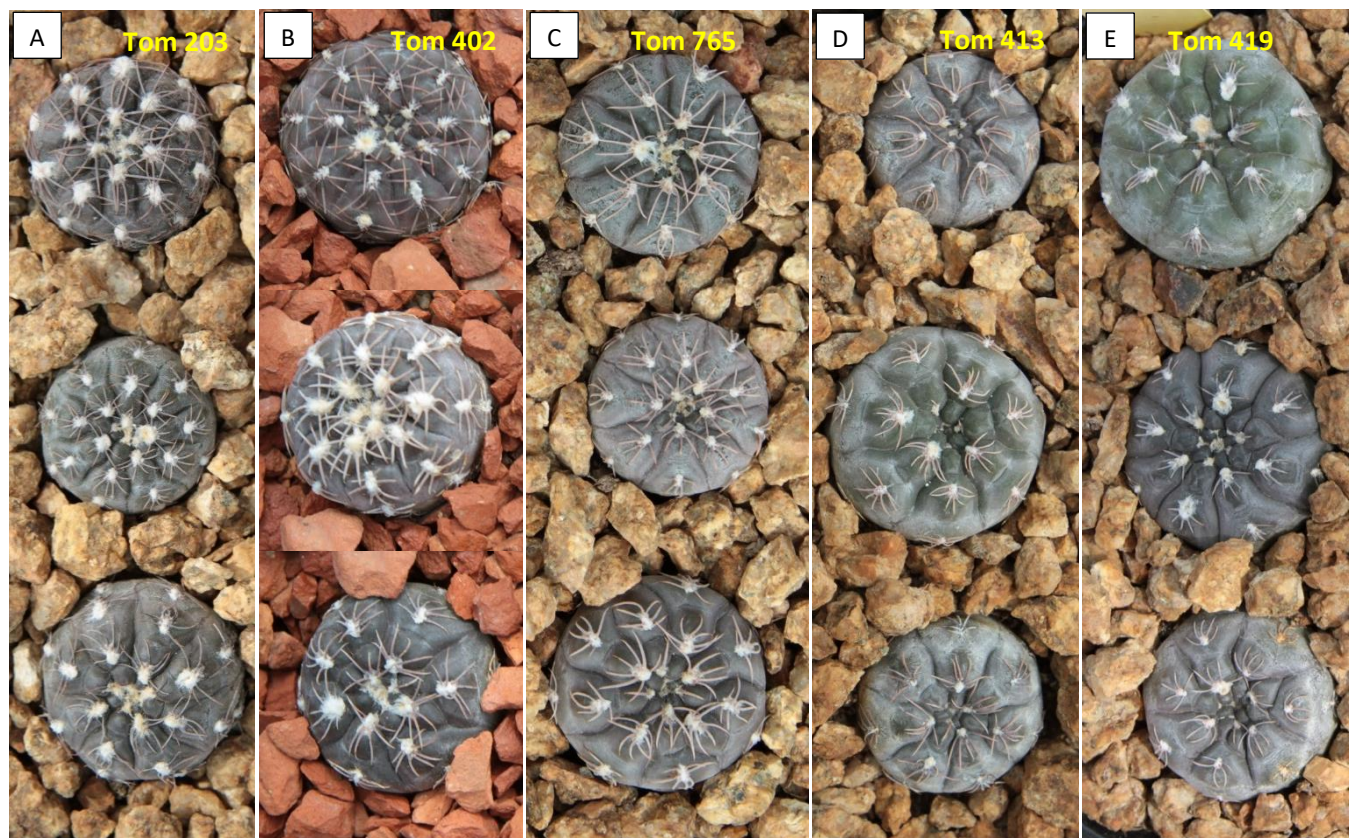


Fig. 24: *G. nataliae* and *G. nataliae* aff.  $2n = 44$ ; **A** *G. nataliae* Tom 07-203, **B** *G. nataliae* Tom 09-402, **C** *G. nataliae* aff. Tom 15-765, **D** *G. nataliae* aff. Tom 09-413, **E** *G. nataliae* aff. Tom 09-419.





Fig. 25: Overview distribution map of all discussed taxa.

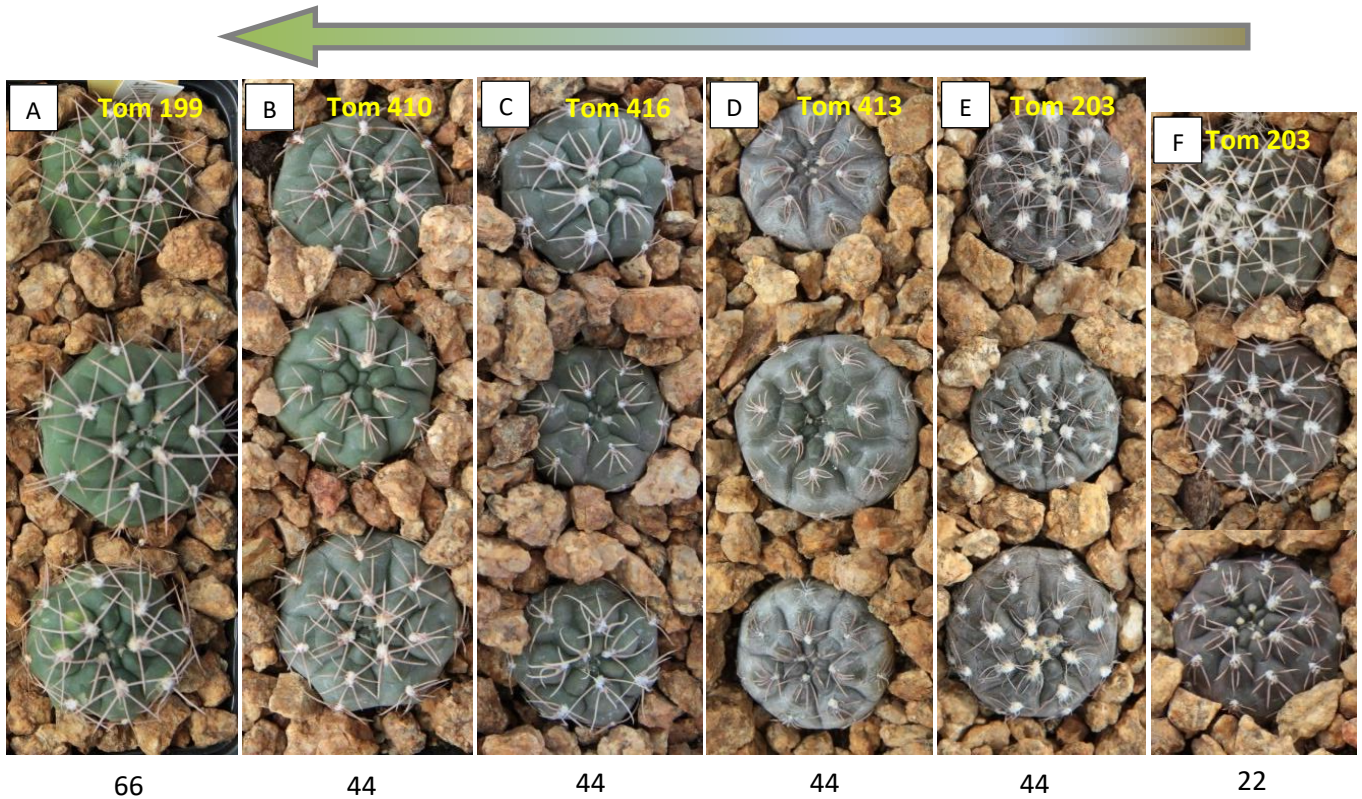


Fig. 26: Comparison of the morphological characteristics of related species; **A** *G. sutterianum* Tom 07-199, **B** *G. morroense* Tom 09-410, **C** *G. morroense* Tom 09-416, **D** *G. nataliae* aff. Tom 09-413, **E** *G. nataliae* Tom 07-203, **F** *G. lukasikii* subsp. *emilii* ( $\times$  *G. nataliae*?) Tom 07-203; pictures below indicate ploidy level =  $2n$ .

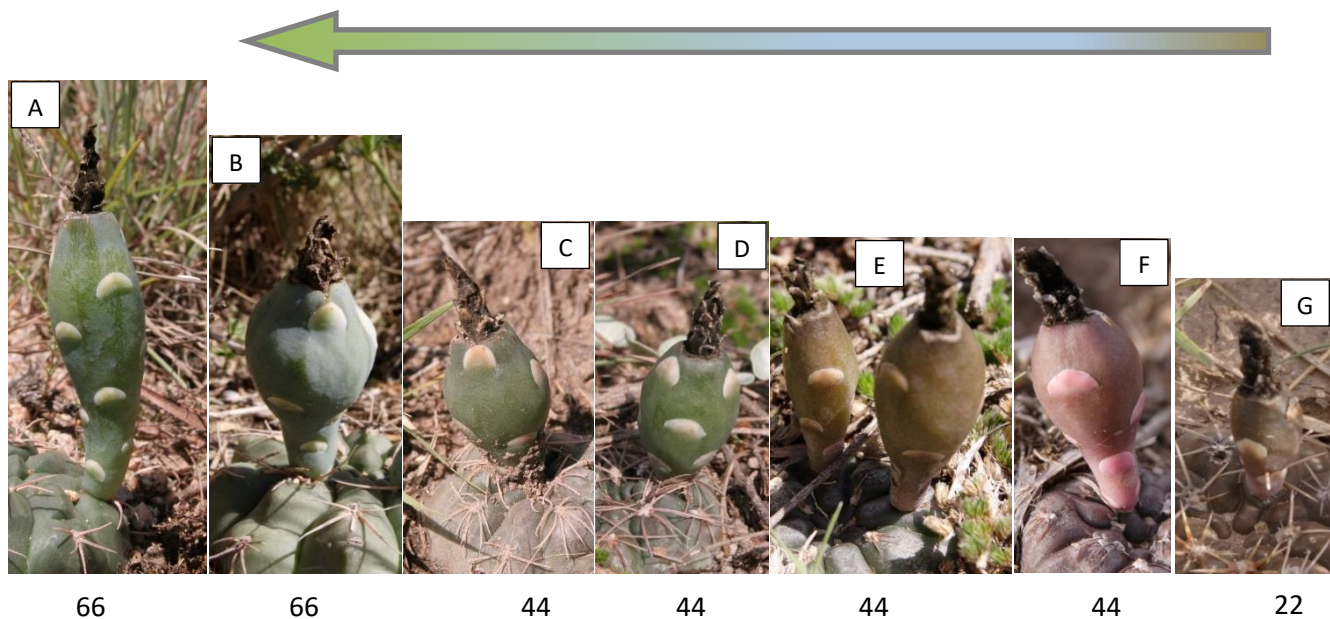


Fig. 27: Comparison of fruits of related species; **A-B** *G. sutterianum*, **C-D** *G. morroense*, **E-F** *G. nataliae*, **G** *G. lukasikii* subsp. *emilii*; below the pictures the ploidy level =  $2n$  is indicated.

## CONCLUSION

Finally, it can be stated that almost every population of *G. morroense* has its own variability and can be considered as a phylogenetically very young species. Higher variability range can correspond with higher ploidy level, as already noticed. *G. morroense* is tetraploid ( $2n = 4x = 44$ ) and an influence of other species growing on the area border or directly inside the area has taken place in a long process of hybridogenesis.

On the edges of the distribution areas of both taxa, populations with mixed or intermediate phenotypes can be found. This phenomenon corresponds with a recent development of such populations and hybridogenesis often influences both species. Concerning *G. morroense*, this condition could be recorded in its north western (South of Las Chacras and eastern areas with *G. sutterianum* - line between villages Potrerillos and Naschel). Populations related to *G. sutterianum* (but without confirmed ploidy level yet) have also been studied inside of the Sierra Yulto, South of Cerro Blanco. In the southwestern area studies were carried out with *G. poeschlii* (between La Toma and Saladillo). The influence of diploid genotypes (*G. lukasikii* or *G. borthii*) on the genotype of *G. morroense* has not been reported yet, although we know about such influence in the case of *G. fischeri* in an area south of San Luis City and *G. poeschlii* in an area between La Toma and Saladillo, close to Loma del Medio. Hybridogenesis with the earlier flowering *G. lukasikii* is much less possible; nevertheless, it may occur thanks to later or second flowering time. This was confirmed by collecting a hybrid fruit from *G. lukasikii* subsp. *emilii* (San Isidro) in mid-January. Raised seedlings undoubtedly show signs of hybridisation with *G. nataliae* which grows syntopically in this habitat (fig. 26 F).

Table 1: List of collections displayed in this study.

| Collection no. |   | species                                  | location |   | altitude | 2n |
|----------------|---|--|----------|---|----------|----|
| Tom 15-763     | 1 | <i>G. sutterianum</i>                    | San Luis | Piscu Yaco, Embalse                                     | 1050     | 66 |
| Tom 09-404     | 2 | <i>G. sutterianum</i>                    | San Luis | 6 km S of Villa de Praga                                | 1083     | 66 |
| Tom 09-143     | 1 | <i>G. sutterianum</i>                    | San Luis | 9 km S of Villa de Praga                                | 1105     | 66 |
| Tom 09-405     | 1 | <i>G. sutterianum</i> aff.               | San Luis | R 40, 2.5 km branch from R 2 to Tilisarao               | 1096     | -  |
| Tom 09-406     | 1 | <i>G. sutterianum</i> aff.               | San Luis | 2 km N from R 2 on branch to San Felipe                 | 1081     | -  |
| Tom 09-420     | 1 | <i>G. sp. green</i> cf. <i>morroense</i> | San Luis | Sierra Yulto, Cerro Blanco, West side                   | 861      | -  |
| Tom 09-421     | 2 | <i>G. sp. green</i> cf. <i>morroense</i> | San Luis | Sierra Yulto, S of Cerro Blanco, from estancia          | 847      | -  |
| Tom 07-205     | 1 | <i>G. sutterianum</i>                    | San Luis | E of Las Chacras  | 1108     | 66 |
| Tom 07-199     | 1 | <i>G. sutterianum</i>                    | San Luis | N of San Felipe, Sierra San Felipe, near embalse        | 860      | 66 |
| Tom 07-230     | 1 | <i>G. sutterianum</i>                    | San Luis | Los Alanices (3 km N of Puerta Colorada)                | 834      | 66 |
| Tom 06-181     | 2 | <i>G. sutterianum</i>                    | San Luis | La Punilla  | 962      | 66 |
| RER 432        |   | <i>G. morroense</i>                      | San Luis | Sierra del Morro, Cerro Guanaco del Morro               | 1060     | 44 |
| Tom 06-173     | 1 | <i>G. morroense</i>                      | San Luis | La Toma Vieja   | 920      | 44 |
| Tom 07-179     | 2 | <i>G. morroense</i>                      | San Luis | La Esquina  | 1070     | 44 |
| Tom 07-196     | 1 | <i>G. morroense</i>                      | San Luis | San Jose del Morro                                      | 1000     | 44 |
| Tom 07-198     | 1 | <i>G. morroense</i>                      | San Luis | R 2, 17 km N of La Toma, S of Paso Grande               | 990      | 44 |
| Tom 07-207     | 1 | <i>G. morroense</i>                      | San Luis | Cruz Brillante  | 1320     | 44 |
| Tom 07-208     | 1 | <i>G. morroense</i>                      | San Luis | Cerros del Rosario, El Mollecito                        | 1116     | 44 |
| Tom 07-209     | 1 | <i>G. morroense</i>                      | San Luis | NW of Cañada San Antonio                                | 1056     | 44 |
| Tom 07-210     | 1 | <i>G. morroense</i>                      | San Luis | S of Los Membrillos → Cañada del Juncal                 | 1156     | 44 |
| Tom 07-211     | 1 | <i>G. morroense</i>                      | San Luis | 1 km N of Los Membrillos                                | 1245     | 44 |
| Tom 09-407     | 1 | <i>G. morroense</i>                      | San Luis | 1.5 km on branch from R 2 to Salado Chico               | 1061     | 44 |
| Tom 09-408     | 2 | <i>G. morroense</i>                      | San Luis | 2 km N of Paso Grande                                   | 1031     | 44 |
| Tom 09-409     | 1 | <i>G. morroense</i>                      | San Luis | Cerros del Rosario, S of Paso Grande                    | 1001     | 44 |
| Tom 09-410     | 1 | <i>G. morroense</i>                      | San Luis | Estancia La Crucesita                                   | 989      | 44 |
| Tom 09-411     | 1 | <i>G. morroense</i>                      | San Luis | 2 km from R 2, branch to Los Vertientes                 | 1059     | 44 |
| Tom 09-412     | 1 | <i>G. morroense</i>                      | San Luis | R 2, X to Los Vertientes                                | 986      | 44 |
| Tom 09-414     | 1 | <i>G. morroense</i>                      | San Luis | Sierra del Morro, Cerro Guanaco del Morro               | 1060     | 44 |
| Tom 09-415     | 2 | <i>G. morroense</i>                      | San Luis | Cerro Guanaco del Morro, 2.2 km E of Tom 414            | 1094     | 44 |
| Tom 09-416     | 1 | <i>G. morroense</i>                      | San Luis | hill below Cerro Guanaco del Morro, near village        | 1110     | 44 |
| Tom 09-203     | 1 | <i>G. nataliae</i>                       | San Luis | R 8a, W of San Isidro                                   | 927      | 44 |
| Tom 09-394     | 1 | <i>G. nataliae</i> aff.                  | San Luis | N of Estancia Maria del Carmen                          | 879      | 44 |
| Tom 09-396     | 1 | <i>G. nataliae</i> aff.                  | San Luis | La Cienaga - La Casilla                                 | 913      | 44 |
| Tom 09-397     | 1 | <i>G. nataliae</i>                       | San Luis | W of La Sola  | 950      | 44 |
| Tom 09-400     | 1 | <i>G. nataliae</i> aff.                  | San Luis | Estancia La Noria, Pampa de San Martin                  | 960      | 44 |
| Tom 09-402     | 1 | <i>G. nataliae</i>                       | San Luis | 2 km SE of Pozo del Espinillo                           | 944      | 44 |
| Tom 09-413     | 1 | <i>G. nataliae</i> aff.                  | San Luis | La Toma - Los Morillos                                  | 963      | 44 |
| Tom 09-419     | 1 | <i>G. nataliae</i>                       | San Luis | Sierra Yulto, on branch from R 148 to Coronel Alzogaray | 748      | 44 |

## FIGURES

Fig. 2, 4, 5 B, 7 A by Radomír Řepka; fig. 7 C by Lumír Král; fig. 9, 19, 21, 23, 25 by Mario Wick. All other figures by the author.

## ACKNOWLEDGMENT

I would like to express my thanks to Doc. Radomír Řepka, PhD for his help in field work, providing pictures and further unpublished data mentioned in this study, to Jaroslav Procházka, Martin Tvrdlík and Lumír Král for their field work and to Dr Mario Wick for his dedicated help in creating maps and the final layout of this paper.

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## Annex

## **Annex: Description of the flow cytometry method used**

PLANT CYTOMETRY SERVICES

tel.: +31 735 475 546

P.O. Box 299

fax.: +31 735 477 181

5480 AG Schijndel

E-mail: info@PlantCytometry.nl

The Netherlands

### **PLOIDY ANALYSIS WITH FLOW CYTOMETRY:**

#### ISOLATION of NUCLEI

Seeds were analysed using *Ophiopogon planiscapus* 'Niger' as internal standard. 10 seeds and 50-100 mg of the internal standard were "chopped" with a sharp razor blade in an ice-cold DNA buffer, in a plastic petri dish.

DNA buffer modified after:

Arumuganathan, K.; Earle, E. D. (1991): Estimation of Nuclear DNA Content of Plants by Flow Cytometry. - *Plant Molecular Biology Reporter*, Vol 9(3), p. 229-233.

5 mM Hepes

10 mM Magnesium sulphate heptahydrate

50 mM Potassium chloride

0.2 % Triton X-100

0.1 % DTT (Dithiothreitol)

1.0 % PVP-40

2 mg/litre DAPI

pH 7.5

DAPI is used as a fluorescent dye which selectively complexes with double-stranded DNA to give a product that fluoresce at 465 nm. DAPI has specific DNA-binding properties with preference for adenine-thymine (AT)-rich sequences. After chopping, the buffer (ca. 2 ml), containing cell constituents and large tissue remnants, is passed through a nylon filter of 50 µm mesh size.

#### FLOW CYTOMETRY

The solution with stained nuclei is send through the flow cytometer. The fluorescence of the stained nuclei, passing through the focus of a light beam from a high-pressure mercury lamp, is measured by a photomultiplier and converted into voltage pulses. These voltage pulses are electronically processed to yield integral and peak signals and can be processed by a computer. When the samples are run with the appropriate filter-settings for excitation and emission, DNA histograms can be produced.

#### MATERIAL

Flow cytometer: Sysmex, Cube with a UV High power LED (365)

Objective: 40 x N.A. 0.8 air (Partec)

Filter combination with DAPI:

Dichroic mirrors: TK 420A

Emission-filter: GG 435E

## New localities concerning the distribution of *Gymnocalycium kuehhasii* Neuhuber et Sperling

### Reiner Sperling

Kamp 2, 33154 Salzkotten (Germany)

E-mail: [reiner-sperling@web.de](mailto:reiner-sperling@web.de)



### Thomas Strub

Hözlstraße 23, 4102 Binningen (Switzerland)

E-mail: [thomas.strub@kabelbinnigen.ch](mailto:thomas.strub@kabelbinnigen.ch)



### ABSTRACT

Since the first description of *Gymnocalycium kuehhasii* in 2008 a lot of new insights into genotype as well as distribution of this species could be obtained.

The northern part of the province Córdoba and the adjacent region of the province Santiago del Estero are home to many of the subgenus's taxa, which could not be attributed unambiguously for a long time. *Gymnocalycium kuehhasii* was also not recognised as a separate species by some of the experts, but considered to be synonymous with *G. robustum* Kiesling, Ferrari & Metzger. Not only due to field studies, but also because of investigation of the chromosome sets of numerous taxa is a reliable new approach possible now. Here the distribution of *G. kuehhasii* with two new subspecies shall be presented.

### KEYWORDS

*Cactaceae*, *Gymnocalycium*, *affine*, *amerhauseri*, *alena*, *campestre*, *erinaceum*, *frankianum*, *robustum*, *kuehhasii*, *kuehhasii* subsp. *corneuspinum*, *kuehhasii* subsp. *incurvatispinum*, *first description*.

### TOPOGRAPHY UND HABITAT DIVERSITY

From a geological point of view the area north of the town Villa Tulumba (province Córdoba, Argentina) up to Sumampa (province Santiago del Estero, Argentina) comprises hilly highlands with a very old geological history. Its altitude is slightly above 800 m. a. s. l. and the northern part is formed by the Sierra de Sumampa.



Map 1: Topography of the region.

Granite can frequently be found in the whole area, often heavily decayed and hardly preserved as a rock formation.

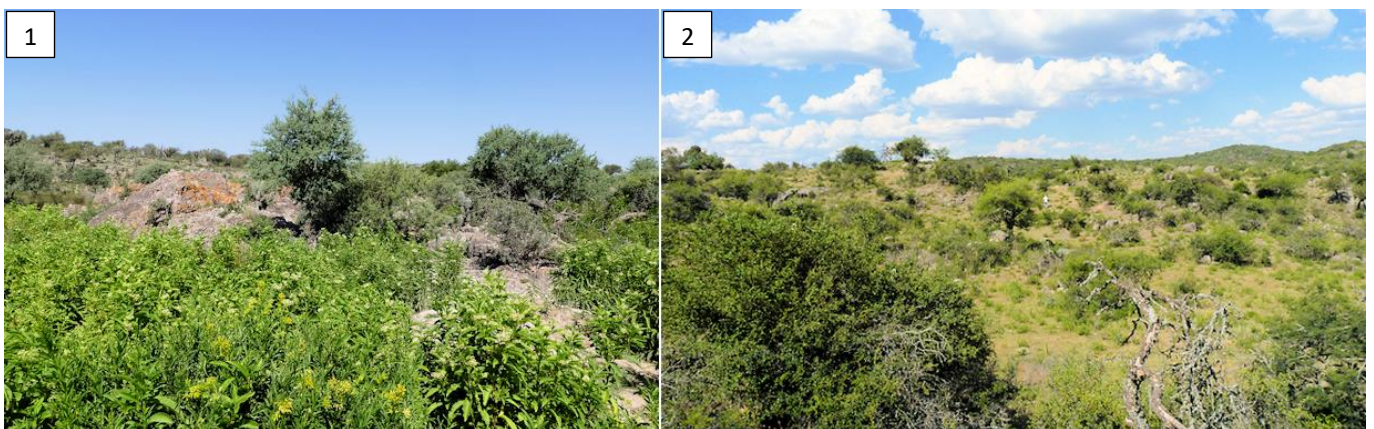


Fig. 1-2: Habitats in the Sierra de Sumampa.

One of the few rock formations, known as Cerro Colorado, can be found further south. This formation, however, consists of red sandstone.



Fig. 3: Cerro Colorado is a part of the mountain range consisting of red sandstone, which rises to somewhat above 800 m. a. s. l.

A vast alluvial region with the Mar Chiquita (79 m. a. s. l.) borders on the Sierra de Sumampa in an eastern direction. To the west the region also gets flatter, forming a plateau interspersed with granite hills of 500-650 m. a. s. l.

Grasslands with sparse bushes, often interspersed with palm trees of the species *Trithrinax campestris*, prevail on the plains and plateaus, in valleys and depressions, which are filled with sediment and decayed rock. The rocky and hilly areas are covered with either sparse or almost impenetrable bushes (mostly *Acacia*), depending on altitude and amount of precipitation. In a northern direction towards Santiago del Estero Chaco vegetation can be found to some extent. A part of the rocky glades in the whole area are to a large extent covered with spike moss (*Selaginella*). This diversity is the reason why the area provides natural habitats for many cacti. Thus *Gymnocalycium* from the subgenera *Muscosemineum* (*G. schickendantzii* (F. A. C. Weber) Britton & Rose), *Trichomosemineum* (*G. quehlianum* (F. Haage ex Quehl) Vaupel ex Hosseus), *Scabrosemineum* (*G. prochazkianum* subsp. *simplex* Řepka) as well as *Gymnocalycium* (*G. kuehhasii* Neuhuber & Sperling, *G. affine* Řepka, *G. campestre* Řepka, *G. alenae* Kulhánek and *G. frankianum* Rausch ex H. Till & Amerhauser) can be found. The accompanying vegetation is composed, among others, of the cactus genera *Lobivia* (*L. aurea*, *L. aurea* subsp. *fallax*), *Acanthocalycium* (*A. spiniflorum*), *Stetsonia* (*S. coryne*), *Trichocereus* (*T. lamprochlorus*), *Harrisia* (*H. tortuosa*), *Opuntia* (*O. salmiana*, *O. quimilo*, *O. sulphurea* aff.) and *Cleistocactus* (*C. baumannii*). These are predominantly heat tolerant or thermophile taxa.



## HISTORY AND TAXONOMY

The subgenus *Gymnocalycium* has given an impact to many discussions for some time. In 2007 Hans Till and Helmut Amerhauser assigned some of the native species (apart from *G. alenae* all the taxa named above were already known at that time, though without names) to *Gymnocalycium quehlianum* (sensu Till), thus to *Gymnocalycium robustum* (sensu Kiesling, Ferrari et Metzinger) (Till & Amerhauser 2007). They also regarded *G. leptanthum* (Spegazzini) Spegazzini as a new subspecies belonging to this taxon and placed *G. calochlorum* (Boedeker) Y. Ito and *G. terweemeanum* (Teuq ex Duursma) Borgmann & Piltz also there as a variety. Their contribution is based on a description by Spegazzini published in 1925, which, from a present-day point of view, should have been emended. The varieties *calochlorum* and *terweemeanum* consequently assigned to that taxon cannot be accepted as varieties in the authors' opinion. The first one belongs to the *capillense*-group and the second one is a 1997 (Piltz & Borgmann) bibliographic rediscovery of a plant that had been missing for almost 100 years. There is no offspring, just an old monochrome photograph. It might be assumed that it shows a taxon of the subgenus *Gymnocalycium*. The other pictures in the article (habitat and cultivation) are not very helpful and rather illustrate the predicament of correctly and comprehensibly assigning plants of the subgenus *Gymnocalycium* from that region to a taxonomic system.

Furthermore, *Gymnocalycium frankianum* Rausch ex H. Till & Amerhauser (collection Walter Rausch WR 722) from the Sierra de Guasayan was described with an indication that this species also occurs in the Sierra de Sumampa (Till & Amerhauser 2007). The taxonomic rank of *G. frankianum* will not be discussed here. However, it is clearly distinct from *Gymnocalycium kuehhasii* and, apart from the Sierra de Guasayan as mentioned by the author, it also grows in the northern Sierra de Sumampa, only a few kilometres away from *Gymnocalycium kuehhasii*.

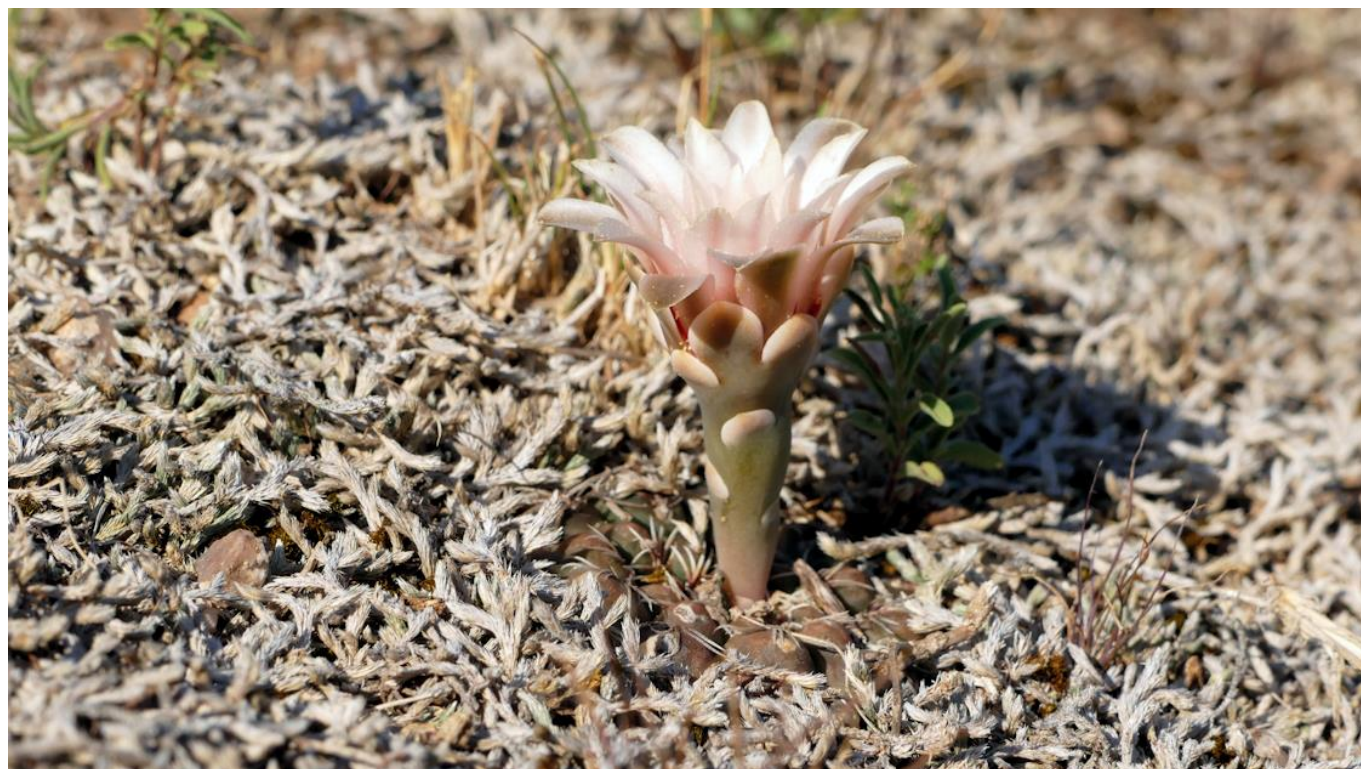


Fig. 4: *G. frankianum* aff. near Villa Ojo del Agua (SPE 374-177-18).

In 2010 Řepka described *Gymnocalycium affine* in “Cactus and Succulent Journal”. These are flat, brown plants with fine spines from the rather meadow-like habitats in the northern Córdoba region. They occur partly sympatrically with *G. kuehhasii* and were mentioned as undefined species in the first description of *G. kuehhasii*.



Fig. 5: *G. affine* (SPE 359-170-18, TS 1406) growing near San Miguel together with *G. kuehhasii*.

In another paper of the year 2015 Řepka described *Gymnocalycium campestre* (fig. 6).

Moreover, the fairly extensive paper differentiates between *G. campestre*, *G. kuehhasii*, *G. amerhauseri*, *G. erinaceum* and *G. robustum* (including distribution map), supported by analyses of ploidy levels.

Thus, *Gymnocalycium campestre*, *G. robustum* and *G. affine* show a tetraploid set of chromosomes ( $2n = 44$ ), while *G. kuehhasii*, *G. erinaceum* and *G. amerhauseri* have a diploid one ( $2n = 22$ ). This difference is significant because diploid and tetraploid species normally either do not crossbreed or are not able to produce fertile offspring (apart from the possibility of autopoloidisation and thus achieving fertility of the resulting  $2n = 66$ ).

Consequently, some scientists' opinion that *G. kuehhasii* must be regarded as synonymous with *G. robustum* cannot be maintained. A relationship could rather be found with *G. erinaceum* or *G. amerhauseri*. These two species, on the other hand, were sufficiently distinguished from others by Řepka (2015). In the authors' opinion the latter two species are from another aggregation.



Fig. 6: *G. campestre* near La Majadilla (SPE 81-33-13).



Fig. 7: *G. erinaceum* at the type locality near Sauce Punco (SPE 82-34-13, TS 612).



Fig. 8: *G. kuehhasii* near San Miguel (SPE 360-170-18, TS 1407).

In the journal “Schütziana” Kulhánek (2017) described *Gymnocalycium alenae* (fig. 9), which also belongs to the subgenus *Gymnocalycium*. There he refers to a possible relationship with *Gymnocalycium tanningaense* Piltz. *G. alenae*'s type locality is a few kilometres southeast of San Francisco del Chañar and it is therefore another name in the region dealt with here. Including *G. tanningaense* is remarkable because the distance to the type locality of this species is approximately 200 kilometres in linear distance and no additional morphologically similar taxa are known in between. From our point of view *G. alenae* must be assigned to another related taxon.

Since the first description of *G. kuehhasii* in 2008 we have obtained a lot of new information, opinions and localities of plants from that region. This has made us further investigate and document northern Córdoba and the distribution areas of the *Gymnocalycia* from the neighbouring Santiago del Estero. In addition to looking at morphological features, seeds of the respective taxa were collected at the habitat for offspring to be further discussed and investigated morphologically and genetically at home (ploidy levels, for methods see appendix to Kulhánek's paper in this edition). Particularly helpful was the investigation of ploidy levels. A part of them were carried out several times and compared with those done by Řepka. The results of the individual species and localities are identical, provided they are comparable. In addition to Řepka's research, *G. alenae*, *G. affine* and *G. frankianum* (locality in the Sierra de Guasayan) and plants similar to *G. frankianum* from the northern Sierra de Sumampa were also included in the investigation.

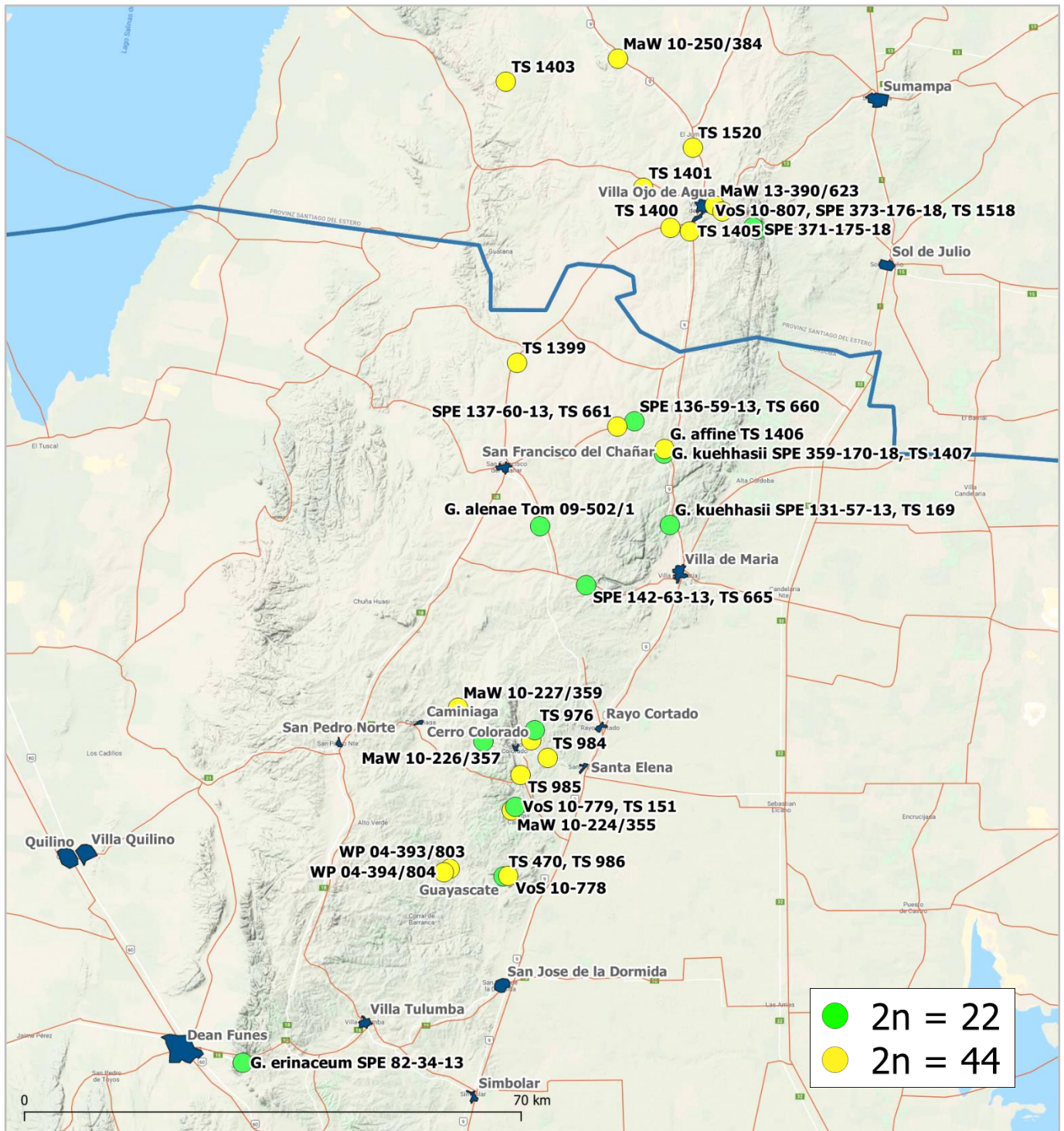


Fig. 9: *G. alenae* grows exposed at the type locality in granite grit rich in quartz (SPE 376-178-18, TS 166).

Table 1: Resume of ploidy level investigation of the taxa from the region northern Córdoba / southern Santiago del Estero.

| Species   | Ploidy level         |
|---|----------------------|
| <i>G. affine</i>                                  | 2n = 44 (tetraploid) |
| <i>G. alenae</i>                                  | 2n = 22 (diploid)    |
| <i>G. amerhauseri</i> subsp. <i>altagraciense</i> | 2n = 22 (diploid)    |
| <i>G. campestre</i>                               | 2n = 44 (tetraploid) |
| <i>G. erinaceum</i>                               | 2n = 22 (diploid)    |
| <i>G. frankianum</i>                              | 2n = 44 (tetraploid) |
| <i>G. frankianum</i> aff.                         | 2n = 44 (tetraploid) |
| <i>G. kuehhasii</i>                               | 2n = 22 (diploid)    |
| <i>G. robustum</i>                                | 2n = 44 (tetraploid) |

As a result, we can differentiate *G. kuehhasii* genetically, too, from *G. affine*, *G. campestre*, *G. frankianum* as well as *G. robustum*, which are, other than *G. kuehhasii*, tetraploid.



Map 2: Subgenus *Gymnocalycium* in northern Córdoba and the adjacent region in the province Santiago del Estero.

In order to reliably establish the distribution range of *Gymnocalycium kuehhasii*, a lot of populations, which seem to us to be phenotypically related with *G. kuehhasii*, were investigated regarding their ploidy levels, although they could not be unambiguously assigned because of some pronounced differences. Among them are, as it is often the case with other species, the border populations of the assumed distribution area. Especially in the southern area around the Cerro Colorado, *G. kuehhasii* aff. ( $2n = 22$ ) can be found growing sympatrically with plants similar to *G. campestre* ( $2n = 44$ ). Here the plants are very similar to each other and extremely variable, so that it is almost impossible to assign the individual plants to the respective species.

## Diploid plants from the subgenus *Gymnocalycium* and their localities in the northern Córdoba region as well as the adjacent area of Santiago del Estero

The northernmost population of plants similar to *G. kuehhasii* known at present occurs in the hills of the Sierra de Sumampa, east of Villa Ojo de Agua. The plants from this locality also have a diploid set of chromosomes, though they differ markedly in some features from plants of the type locality KF93-128/472.

On this occasion once more the first description of *G. kuehhasii* Neuhuber & Sperling from *Gymnocalycium* 21(1): pp. 747-750:

**Typus:** Argentina, Provincia de Córdoba, prope pagum San Miguel, 550-650 m s. m., 16. Enero 1993, leg. F. Kühhas KF93-128/472, Holotypus: CORD (ex WU 1060, flos in liquore alcoholico), Isotypus: WU (Nr. 889 et Nr. 1212, flos in liquore alcoholico; Nr. 2508, fructus siccus); l. c., F. Kühhas KF93-128/472a, Paratypus: WU (Nr. 2172, flos in liquore alcoholico).

**Body** solitary, flat hemispherical, not offsetting, up to 40 mm in height, up to 90 mm in diameter. **Tap root** short, mostly not strong, appearing attached. **Epidermis** in cultivation grey-green to dark grey-green, matt. **Apex** little sunk, armed with spines. **Ribs** -12, running down straight and getting wider at the bottom, dissolved into **protuberances** positioned close to one another with markedly protruding **chins**, separated by deep, later wavy, longitudinal grooves. **Areoles** round, sunk, with a lot of white wool, about 9 mm apart. **Spines** (3-) 4-5 (-7), thin, occasionally somewhat flat, slightly protruding or horizontally close to the body, light horn-coloured, apical almost white, without a dark tip, at the base darker, becoming black with age without greying. No **central spine**. **Flowers** originating at the apex, not odorous, 65-70 mm in length, 40-45 mm in diameter, white to dirty white, bell-shaped, opening only slightly. **Outer petals** white with light rose-coloured or light green mid stripe, partly broad and of light greyish green colour dorsally, light rose-coloured at the base, 23.5 mm long as an average, 6.5 mm wide, lanceolate. **Inner petals** white, 19 mm long, 5 mm wide, narrow lanceolate and pointed. **Flower throat** with thick pericarpel wall, light rose-coloured to rose-coloured, 15 mm high, 9 mm in diameter. The long, narrow **nectar chamber** is light green to white at the base, further up light rose-coloured to light orange, up to 8 mm high, 2-3 mm in diameter. **Filaments** up to 6.8 mm long, mostly light yellow. 1 primary line, 4 mm long, consisting of few stamina whose anthers rest against the middle of the stigma or directly underneath, **filament base** light green to the middle, then white, primary line with a pronounced distance from the 4 (-6) secondary lines which rest tightly against the inside of the throat. Only towards the upper end of the throat the secondary lines strongly curve to the middle with their **anthers** forming a roof above the stigma without touching it. Anthers 1.3 mm long and 0.6 mm wide, yellow. **Style** light green, 11 mm long, 1.2 mm in diameter, protruding far into the ovary, septum to the ovary white and often bent downward. **Stigma** light yellow, 2.5 mm long, 9-11 lobes always below the anthers of the secondary lines. **Ovary** white, 16.5 mm long, 4 mm in diameter. **Pericarp** 22 mm high, 10 mm in diameter, distinctly cone-shaped, dark green, slightly frosted with a grey hue. **Scales** broadly hemispherical, not or only scarcely discernibly curving down at the sides, light green with light rose-coloured edges. **Fruit** about 23 mm long in dry state and up to 12 mm in diameter, green to bluish green, ellipsoid to spindle shaped. Base of the fruit conical, fruit dehiscing vertically, the seeds are squeezed out of the fruit. **Seed** 1.15-1.2 mm long, up to 1.1 mm in diameter, straight towards the HMR or even becoming wider. **Testa** black with mostly

pronounced rounded structures, covered with little brown skin, Hilum-Micropylar-Region (HMR) large, wide drop-shaped, distinctly separated, sunk, brown (subgenus *Gymnocalycium*).

**Home:** Argentina, province Córdoba, region around Villa Maria, 550-600 m. a. s. l., always growing in the shadow of bushes.



Fig. 10: *G. kuehhasii* near San Miguel (SPE 360-170-18, TS 1407).

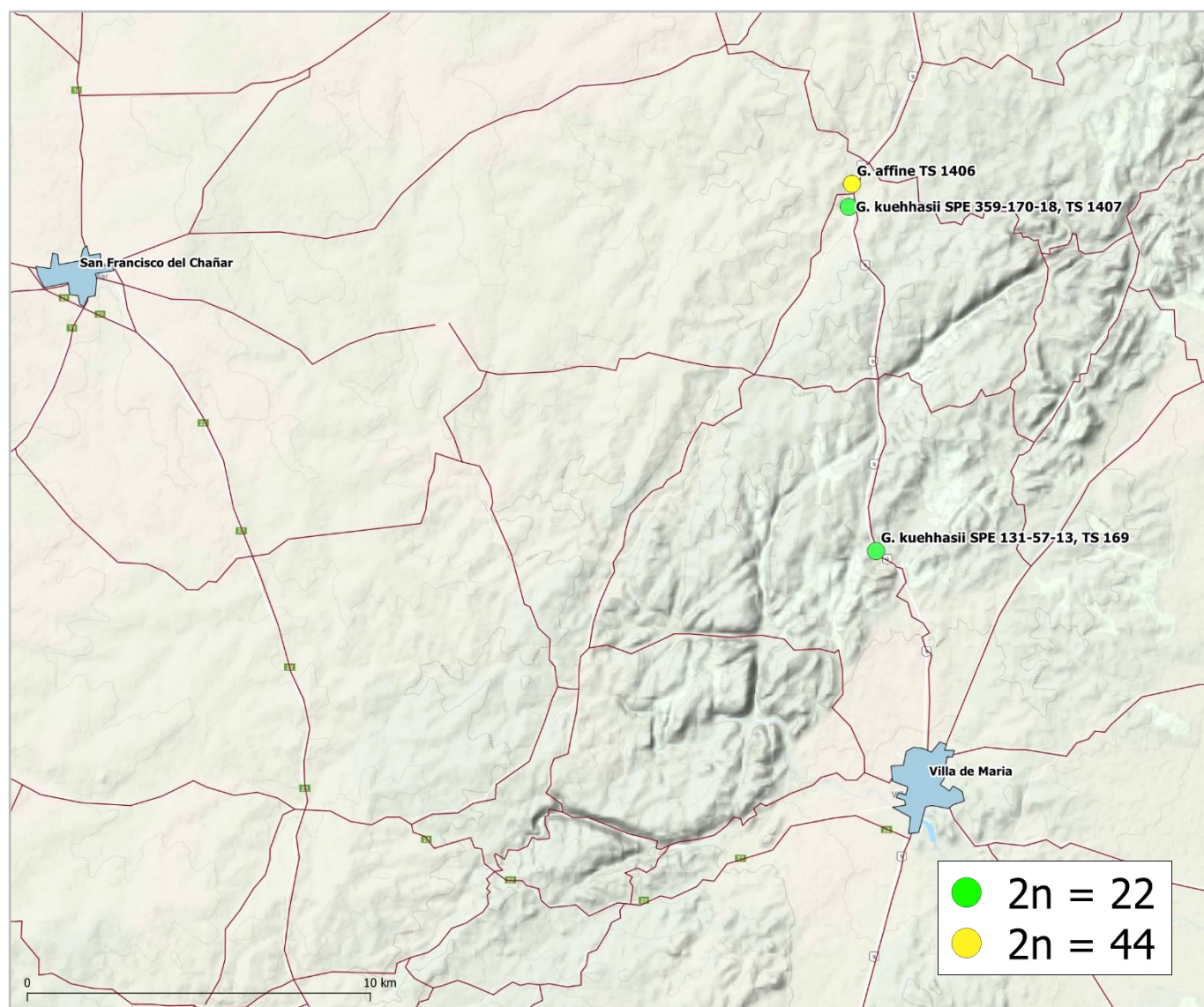
## DISCUSSION

The plant is not comparable with any other taxon occurring in the area. The mostly very dark epidermis, the long, almost white spines within white, woolly areoles and the special position of style and stigma (top edge of the stigma below the lowest anthers of the secondary lines) let us easily recognise *G. kuehhasii*. At the localities of *G. kuehhasii* the occurring *Gymnocalycia* are largely of the seed group *Gymnocalycium*, although they cannot be identified unambiguously due to the lack of a definite description by Spegazzini as well as H. Till & Amerhauser (the latter missed emending their subspecies *leptanthum*). The note "...in nature and without seeing the flower it is difficult to establish if the plant is a *G. quehlianum*, *leptanthum* or even a *G. parvulum* (Speg.) Speg." is, in our opinion, not sufficient as a basis for the description of a species resp. subspecies, as the flower was not described clearly and there is no photograph of a flower section. The plants also occurring in that region, which cannot be assigned to *G. kuehhasii* at present, grow exposed to the sun, they are mostly sunk deeply into the soil and with a brown epidermis caused by sunlight. They possess 9 short, brown spines tight to the body on rounded ribs with



wide protuberances, the flower is rose-coloured, the ovary varies in relation to length of the petals and the stigma is always positioned in the middle of the secondary anther lines. The fruit is clavate and frosted with a greyish blue hue (see fig. 5. In the meantime, the plants were described by Řepka as *G. affine*).

Plants (TS 169, SPE 131-57-13, SPE 356-57-18), which correspond with the type plant KF 93-128/472 as well as its offspring, were found by us (Volker Schädlich, Reiner Sperling, Thomas Strub and Mario Wick) during our joint journey in 2013, approximately 9 kilometres south of San Miguel at an altitude of 480 m. a. s. l. along the RN 9, together with nine! other cactus species from various genera.



Map 3: Localities north of Villa de Maria.



Fig. 11: Locality of *G. kuehhasii* 9 km south of San Miguel.

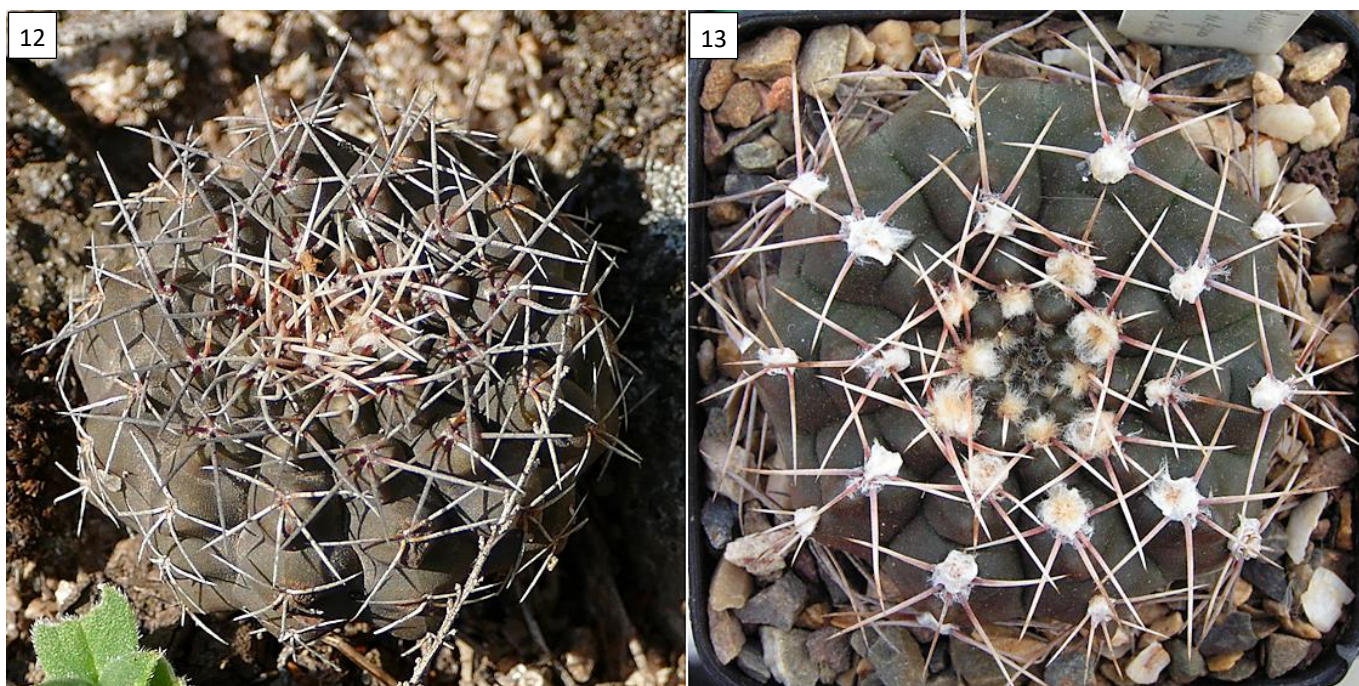


Fig. 12-13: At their locality the plants grow in the shadow of granite blocks (fig. 12) and correspond well with the type KF93 128-472 (F1-offspring) (fig. 13).

A constant companion which also grows at all the other localities of *G. kuehhasii* is *Acanthocalycium spiniflorum*.

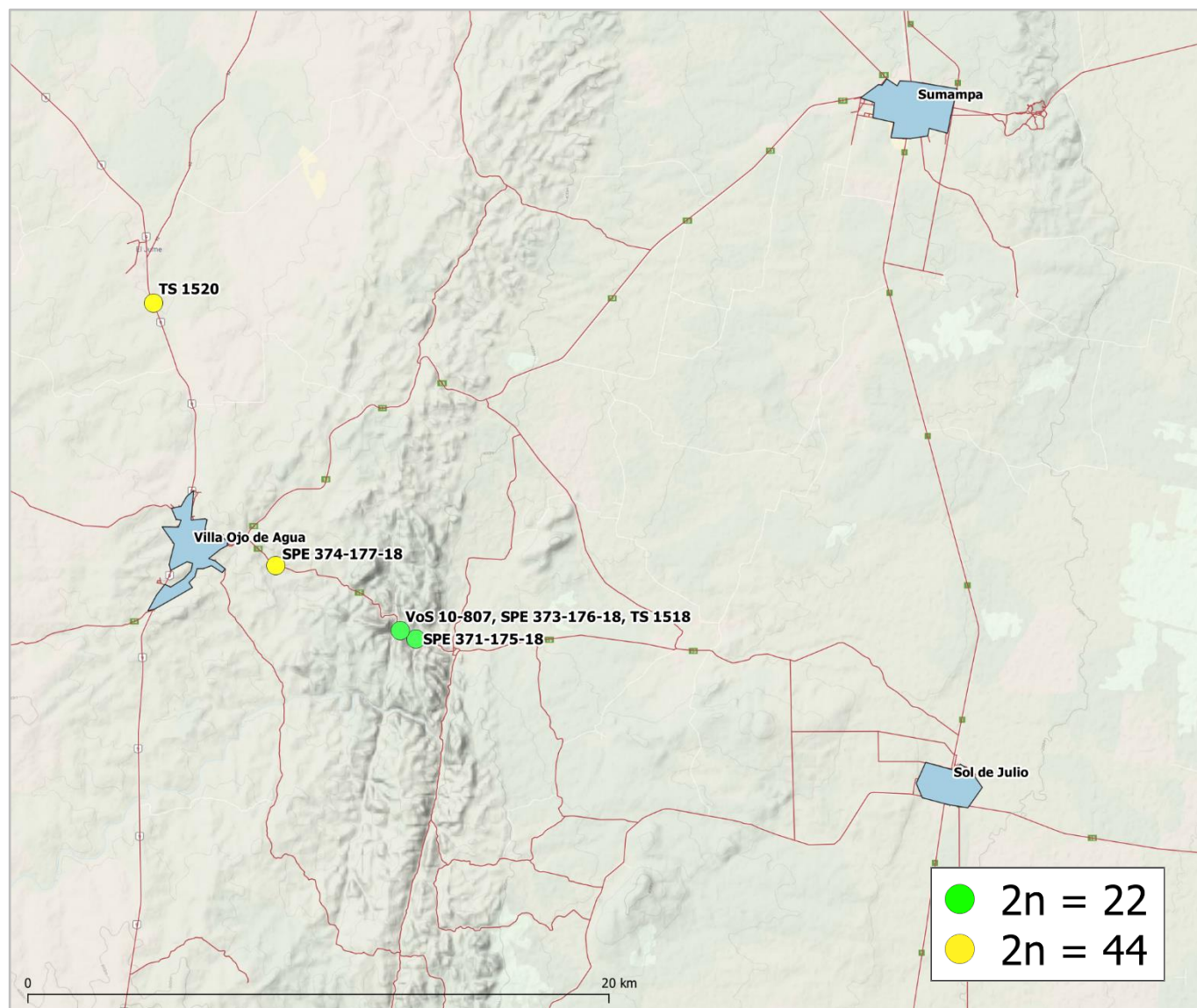


Fig. 14: *Acanthocalycium spiniflorum*.

### Northern occurrence

Compared with the first description and especially with the plants at locality KF93-128/472, the plants from the northern Sierra de Sumampa in the province Santiago del Estero show distinct differences. They grow at somewhat lower altitudes (370-400 m), but also partly in very shaded places underneath bushes. During a common journey in 2010 they were found by Thomas Strub (TS 178), Volker Schädlich (VoS 10-807) and Mario Wick (MaW 10-248/382) due to a hint from Massimo Meregalli, who had discovered the locality the previous day and had transmitted the coordinates via GPS. Further field numbers of this population are TS 1518, SPE 371-175-18, SPE 373-176-18, Tom 12-733/1, VoS 18-2344, VoS 18-2342, LB 5563 and MM 1048.

The **bodies** are green to olive green and possess wide **ribs** with very prominent **protuberances** which are partly slightly flattened laterally. The distances between the **areoles** are longer than those of the type (up to 14 mm) and are covered with yellow felt-like wool. Spination is longer than with the type (up to 15 mm), partly just horn-coloured and only slightly darker at the base, not greying or becoming black with older age. As a rule, they form (4-) 5-7 (-8) **radial spines**, which protrude from the body at an angle of 45 degrees. Typical for the plant is an up to 15 mm long, needle-shaped **central spine** which protrudes at a right angle to the rib. The features referred to here give the plant a cruder, more robust appearance. The **root** is often divided into two to three strong strands which branch further. As this population has a diploid chromosome set ( $2n = 22$ ), too, and is not considerably distinct in seed and flower features as well as the arrangement of spines from the *G. kuehhasii* type, we herewith suggest the rank of a new subspecies of *G. kuehhasii*.



Map 4: The northernmost occurrence known of *G. kuehhasii* is located in the province Santiago del Estero: *G. kuehhasii* subsp. *corneuspinum* (green dots).

***Gymnocalycium kuehhasii* Neuhuber et Sperling subsp. *corneuspinum* Sperling et Strub, subsp. nov.**

Diagnosis

Differs from *G. kuehhasii* subsp. *kuehhasii* by its olive-green body, wider ribs with wider separated areoles, stronger horn-coloured spines and a central spine.

Typification

The plants grow shaded under bushes in grass on stony ground, Argentina, province Santiago del Estero, east of Villa Ojo de Agua (S29°31.179, W63°38.134, altitude: 375 m. a. s. l.). First discovery 17<sup>th</sup> January 2010.

Herbarium material

Cultivated flowering plant grown from seeds collected from the habitat. Holotype: Volker Schädlich VoS 10-807 (Herbarium WU 4117) (fig. 19).

Etymology

The name refers to the horn-coloured spines, which is different from subsp. *kuehhasii*.



Abb. 15: Habitat of *G. kuehhasii* subsp. *corneuspinum*.

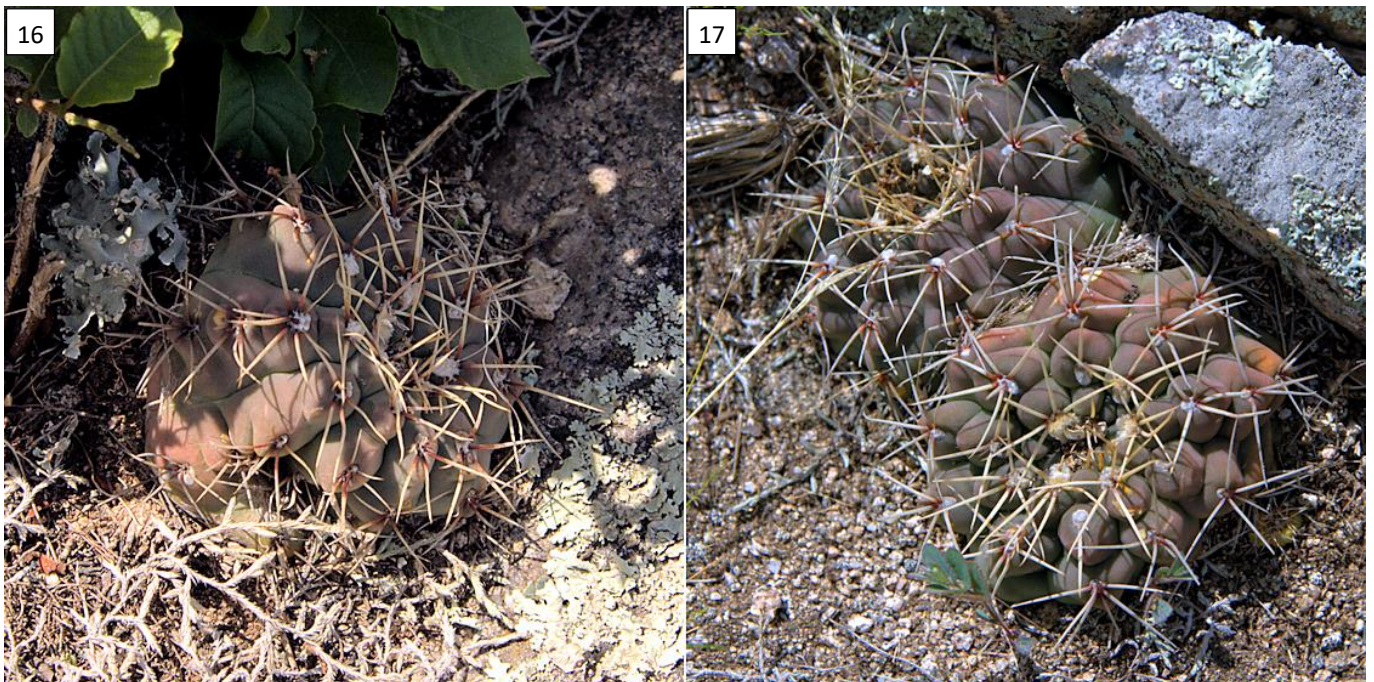


Fig. 16-17: *G. kuehhasii* subsp. *corneuspinum* in Santiago del Estero, VoS 10-807 (photographs: Volker Schädlich).



Fig. 18: *G. kuehhasii* subsp. *corneuspinum* in Santiago del Estero, SPE 371-175-18.

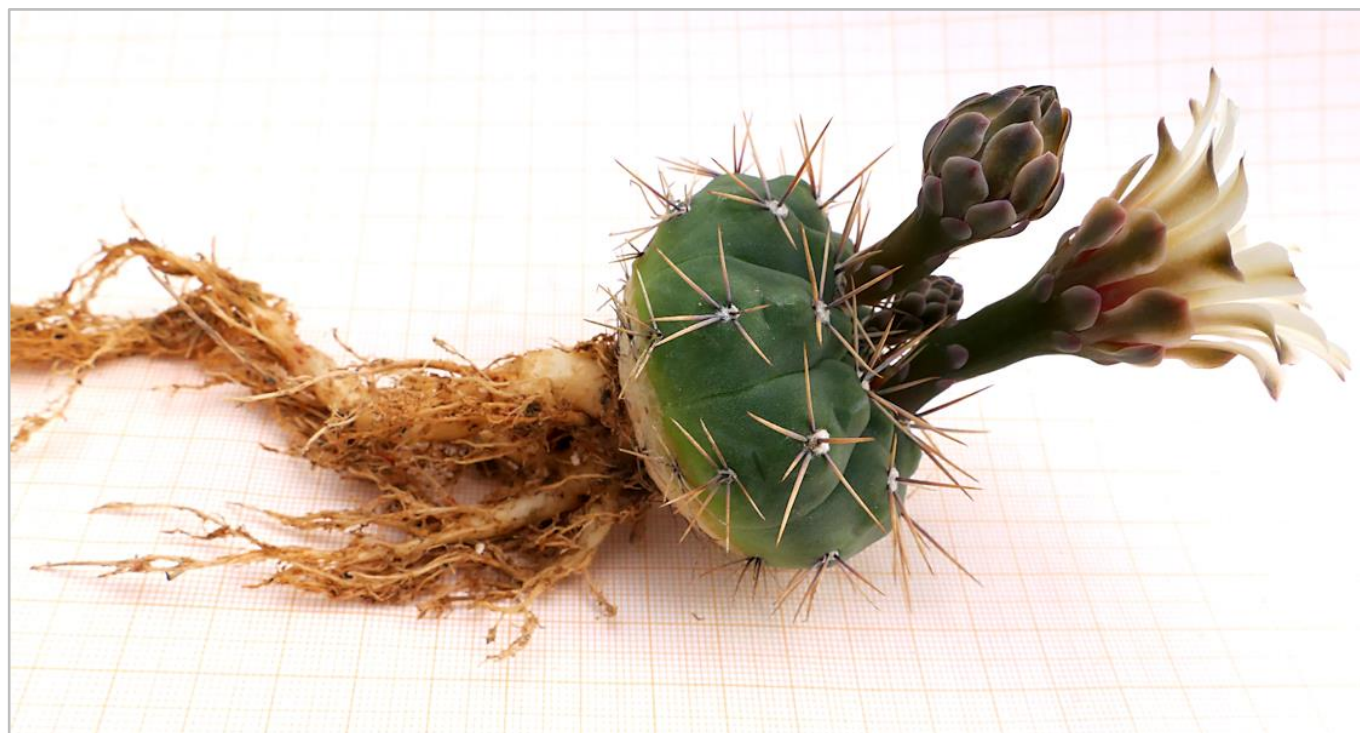


Fig. 19: Holotype of *G. kuehhasii* subsp. *corneuspinum*, VoS 10-807.

## Occurrence west of San Miguel

At the beginning of January 2013, we came across a population 5 km west of San Miguel, which we did not recognise as *Gymnocalycium kuehhasii* in habitat. Only the sympatrically occurring *G. affine* could be identified unambiguously.

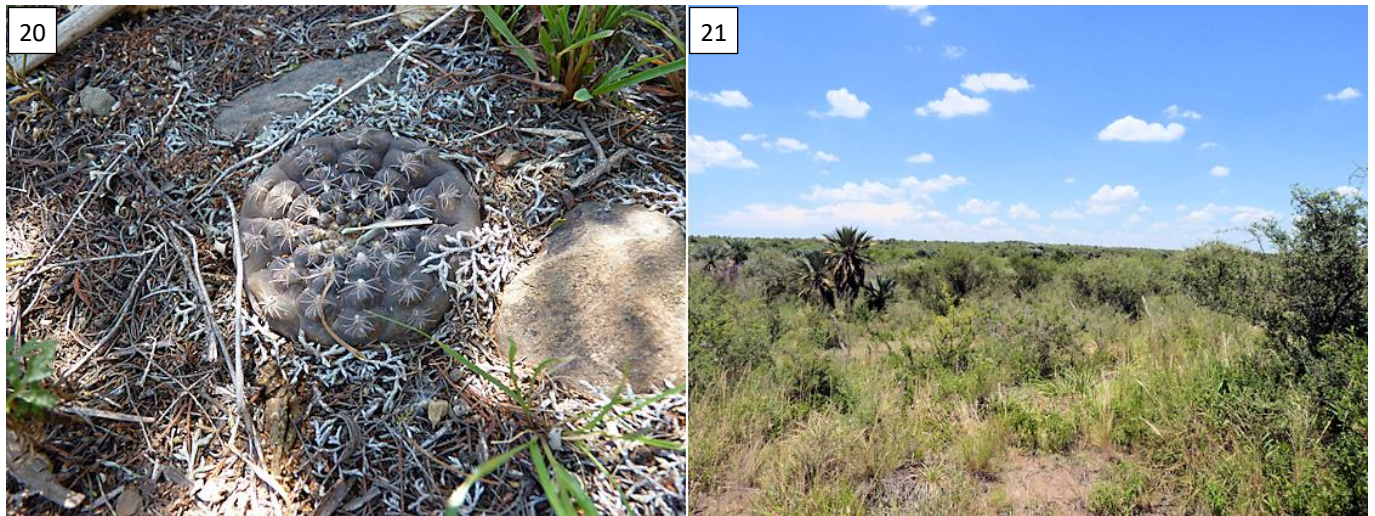
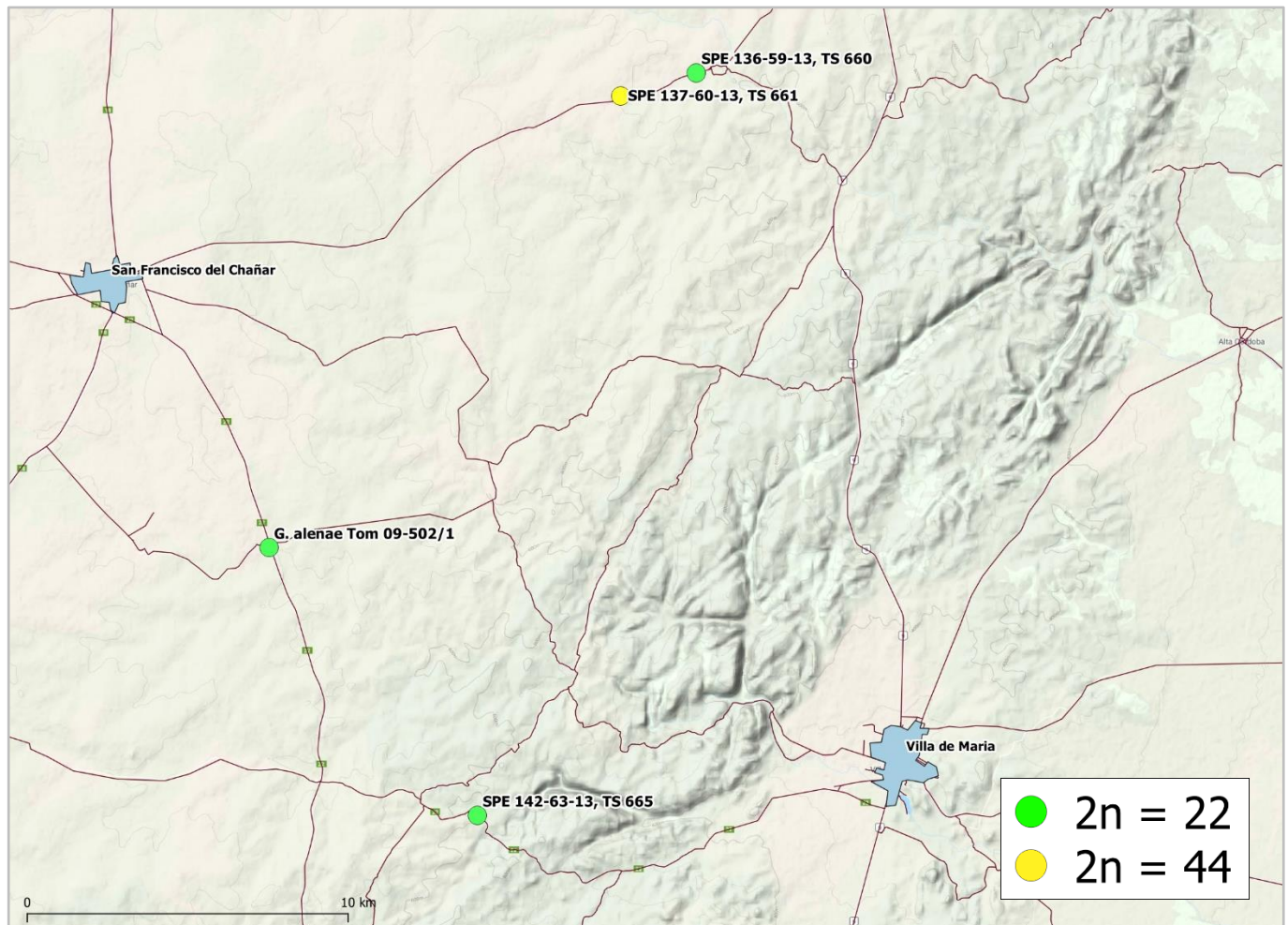


Fig. 20-21: *Gymnocalycium affine* at locality SPE 136-59-13, TS 660.

Fruit and seeds could be found and gathered from both taxa (MaW 10-248/382, SPE 136-59-13, TS 660, VoS 13-1380). The chromosome set of one of the taxa turned out to be diploid.



Map 5: Localities southwest of San Miguel.

The sowings confirmed that two species occur at this locality: *G. affine* and plants similar to *G. kuehhasii*. Even though, the latter strongly differs from the type regarding its spination pattern. The yellowish to almost white, 5-10 radial spines are not radially arranged and cannot always be found in a round areole. The pattern ranges from slightly pectinate to largely pointing downwards. The spines are finer, curved and are often bent close to the body.



Fig. 22: *G. kuehhasii* subsp. *incurvatispinum*, the plant grew completely covered in grass (SPE 136-59-13, TS 660).



Fig. 23: *G. kuehhasii* subsp. *incurvatispinum* with pure white spination.





Fig. 24: *G. kuehhasii* subsp. *incurvatispinum*, plants growing exposed form fewer spines and are strongly reddened.

25 km further south we came across similar plants with a diploid chromosome set as well (MaW 10-252/386, SPE 142-63-13, TS 665, VoS 13-1383). Investigation of offspring as well as analysis of photographs also proved the association with *G. kuehhasii*.



Fig. 25: *G. kuehhasii* subsp. *incurvatispinum* SPE 142-63-13, TS 665.

The plants grew together with *Acanthocalycium spiniflorum*, partly exposed at a completely sunny site. They were rather small, (3-4 cm in diameter), very much shrunk and partly almost burnt. Thus, an identification at the site turned out to be rather difficult in this case, too.



Fig. 26: Direct sunlight is badly tolerated by *G. kuehhasii* subsp. *incurvatispinum* (photograph: Volker Schädlich).

Thomas Strub was able to visit the locality again in 2014 (TS 981) and found further plants in good shape growing shaded under bushes. In nature the bodies are almost black-green and the very fine spines are almost black beyond the apex.



Fig. 27-29: *G. kuehhasii* subsp. *incurvatispinum* at the same locality (TS 981) one year later with plants of various ages.

In cultivation (in the meantime 6-year-old offspring, see fig. 30-33) the plants barely differ from the specimens occurring 25 km to the north. In juvenile specimens of both taxa the almost purely white, fine spination is striking. Only later does a brown foot appear at the base. Body colour is

also somewhat darker in cultivation than with the type. However, at the same age the plants remain smaller and the ribs are less wide. The flower is a little narrower and funnel shaped. The fact that the spines become black with older age in habitat can so far not be observed in cultivation.



Fig. 30-33: 6-year-old offspring of SPE 136-59-13 (fig. 30) and SPE 142-63-13 (fig. 31) TS 660 (fig. 32) and TS 665 (fig. 33). The plants from both localities can hardly be told apart.

***Gymnocalycium kuehhasii* Neuhuber et Sperling subsp. *incurvatispinum* Sperling et Strub subsp. nov.**

**Diagnosis**

Differs from *G. kuehhasii* subsp. *kuehhasii* by a dark black-green body, narrower ribs and especially the arrangement and shape of the spines.

### Typification

The plants grow shaded under bushes in grass on stony ground in the province Córdoba, Argentina, southwest of San Francisco del Chañar (S29°55.053, W63°50.502, altitude: 600 m. a. s. l.). First discovery on 24<sup>th</sup> January 2013.

### Herbarium material

Cultivated flowering plant grown from seeds collected in the habitat, in alcohol. Holotype: Sperling SPE 142-63-2013 (Herbarium WU 4116).

### Etymology

The Name refers to the inwardly curved spines.



Fig. 34: *G. kuehhasii* subsp. *incurvatispinum* Holotype, SPE 142-63-13.

35



36



Fig. 35-36: *G. alenae* (Tom 502/1) (fig. 35), *G. kuehhasii* subsp. *incurvatispinum* (SPE 142-63-13) (fig. 36).



Fig. 37: Flower sections of *G. alenae* (left) and *G. kuehhasii* subsp. *incurvatispinum* (right).

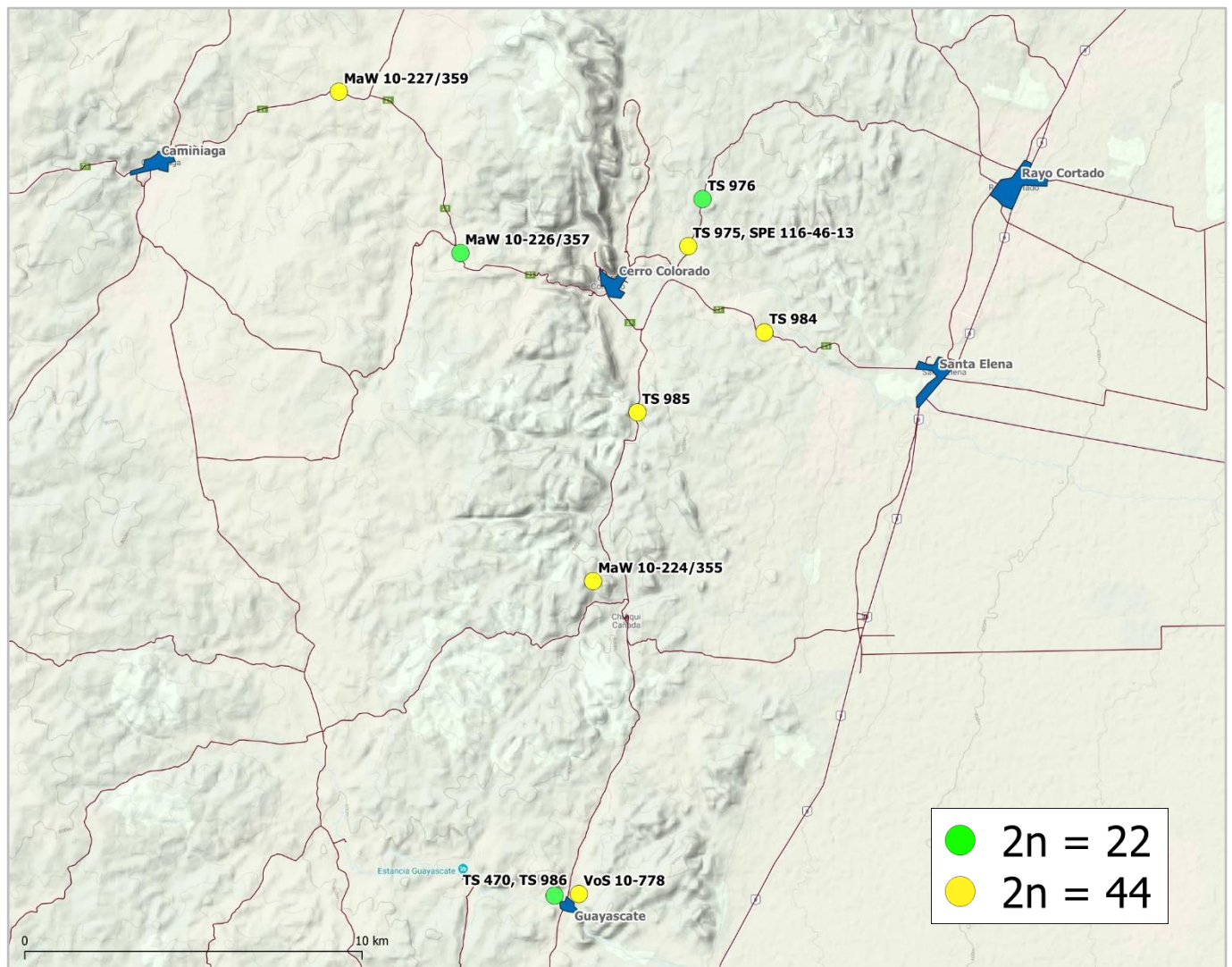
## DISTRIBUTION AND DISCUSSION

*Gymnocalycium alenae* (also  $2n = 22$ ), which grows only 11 km away from the area, cannot be considered as directly related species because there are distinct differences in body, flower as well as seed features. *G. alenae* possesses small, dark green-brown bodies with hardly raised ribs. The protuberances are flat with areoles closer together. Flowers are long funnel-shaped, yellowish on the inside with barely noticeably red throat. The seeds are larger with an oval hilum. *G. alenae* grows completely exposed to the sunlight in habitat. The flowering season starts clearly earlier in the year (early bloomer).

### Southern occurrence

Plants from habitats situated further south around the Cerro Colorado partly belong to *Gymnocalycium kuehhasii* subsp. *incurvatispinum* as well.

However, the habitat conditions change, the countryside becomes more varied. Humus meadows alternate with rocky expanses and dense bush covering. The altitude increases, sandstone formations with the respective sediments change the soil composition in many places.



Map 6: Locations around the Cerro Colorado.



Fig. 38: Habitat southwest of the Cerro Colorado.

*G. affine* does not grow here anymore, instead plants similar to *G. campestre* (tetraploid) as well as *G. quehlianum* (*Trichomosemineum*) often occur even sympatrically.

Differentiation in nature is very difficult, *G. campestre* shows a similar phenotype and seems to occur too in the surroundings of the Cerro Colorado, although some of these plants become very large there and form very pronounced protuberances at old age.



Fig. 39: *Gymnocalycium spec.* TS 985 ( $2n = 44$ ), 8 km north of Churqui Cañada.

Spination is usually longer and stronger than with *G. kuehhasii* subsp. *incurvatispinum*, partly, however, also reduced, depending on the respective habitat conditions. The normally matt body surfaces of both taxa are glossy under ideal growth conditions.

The phenotype of *G. kuehhasii* subsp. *incurvatispinum* can at least to some extent easily be recognised by the darker body colour and typical spine arrangement (in cultivation the features emerge more obviously) and research also confirmed a diploid chromosome set (TS 470, TS 986, TS 976, MaW 10-226/357).





Fig. 40-43: *Gymnocalycium kuehhasii* subsp. *incurvatispinum* TS 986 (fig. 40-41) TS 976 (fig. 42-43).

It requires much more field research and work in the area around the Cerro Colorado to completely depict the distribution area of *Gymnocalycium kuehhasii*.

### ***Gymnocalycium kuehhasii* and its subspecies: comparison and discussion**

According to our present state of knowledge *Gymnocalycium kuehhasii* has a distribution area of about 100 km in a north-south-direction. Whilst it grows in the northern part of the area in almost pure residual soil of granite (subsp. *corneuspinum* and subsp. *kuehhasii*), it occurs in the south and west rather on tertiary sediment from a mixture of rocks (subsp. *incurvatispinum*). The individual populations of the species thus differ noticeably, although they possess common morphological features. All seeds have a cuticula more or less detaching itself from the seed and the seeds are of uniform appearance.



Fig. 44-47: Seed of *G. kuehhasii* subsp. *corneuspinum*, VoS 10-807 (fig. 44), *G. kuehhasii* subsp. *kuehhasii*, SPE 360-170-18 (fig. 45), *G. kuehhasii* subsp. *incurvatispinum* SPE 142-63-13 (fig. 46), *G. kuehhasii* subsp. *incurvatispinum* TS 470, TS 986 (fig. 47).

The flowers do not differ.

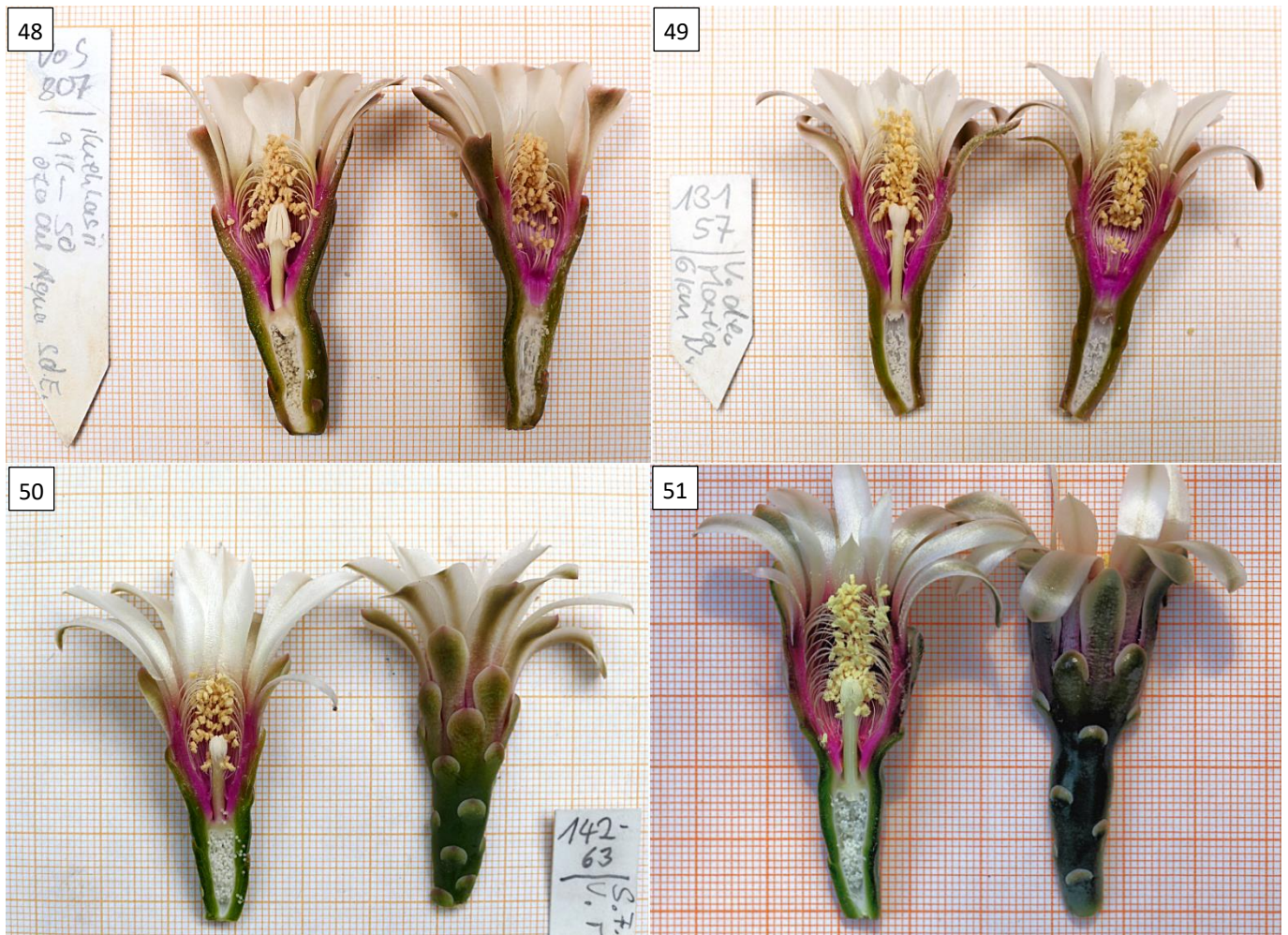


Fig. 48-51: Flower sections of *G. kuehhasii* subsp. *corneuspinum*, VoS 10-807 (fig. 48), *G. kuehhasii* subsp. *kuehhasii*, SPE 131-57-18 (fig. 49), *G. kuehhasii* subsp. *incurvatispinum* SPE 142-63-13 (fig. 50), *G. kuehhasii* subsp. *incurvatispinum*, TS 153 (fig. 51).

Even one-year-old seedlings show differences in size, colour and spination.



Fig. 52: 1-year-old seedlings of the subspecies: *corneuspinum* (left), *kuehhasii* (middle) and *incurvatispinum* (right).

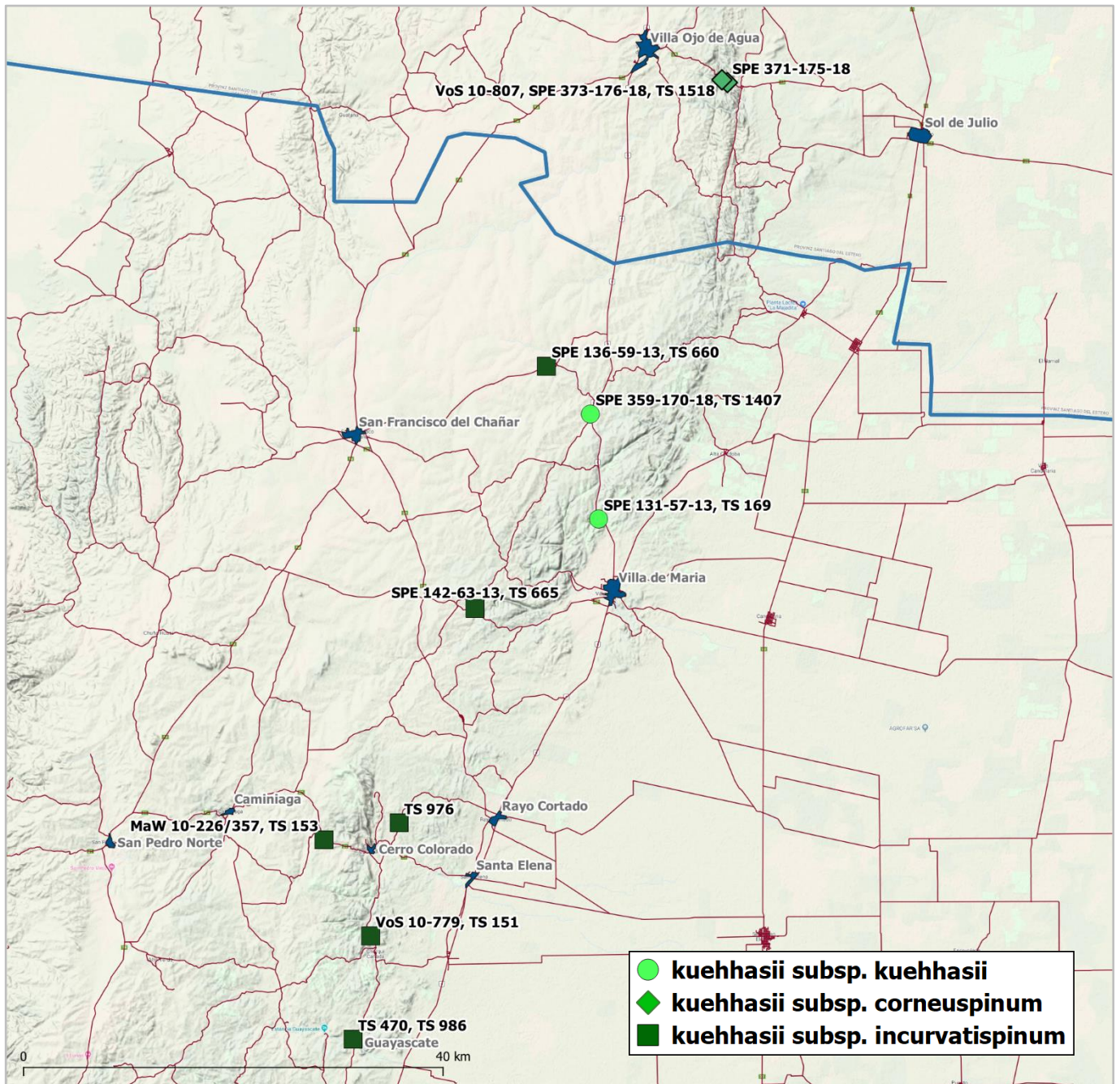
Older plants (6 years old) of the three *G. kuehhasii* species can easily be told apart.



Fig. 53: *G. kuehhasii* subspecies: *corneuspinum* (left), *kuehhasii* (middle) and *incurvatispinum* (right).

Table 2: Comparison of different features

| Features                             | <i>G. kuehhasii</i> subsp. <i>corneuspinum</i>   | <i>G. kuehhasii</i> subsp. <i>kuehhasii</i>   | <i>G. kuehhasii</i> subsp. <i>incurvatispinum</i>   |
|--------------------------------------|--|---|---|
| Body size (6 years, cultivation)     | 55 mm  | 50 mm   | 40 mm   |
| Body colour                          | olive green  | dark green to greyish green   | dark green to black-green   |
| Number of ribs                       | 9-10   | 9-11  | 10  |
| Width of ribs (6 years, cultivation) | up to 22 mm  | up to 17 mm   | up to 14 mm   |
| Distance of areoles                  | about 14 mm  | about 9 mm  | about 9 mm  |
| Areoles                              | round, beige   | round, white  | round to oval, white  |
| Spines                               | up to 16 mm long, horn-coloured, solid, straight, protruding from the body at an angle of up to 45 degrees, almost radially arranged | up to 12 mm long, reddish brown at the base to white at the apex, parallel to the body to slightly protruding, almost radially arranged | 5-10 mm long, fine, white, brown to dark brown at the base, often curved inward to the body, partly directed downward |
| Number of spines                     | 3-(5)-7  | 3-(5)-7   | 5-8   |
| Central spine                        | 1, up to 15 mm long  | 0   | 0   |
| Root                                 | branching  | tap root  | tap root  |
| Seedlings (1-2 years)                | up to 20 mm in size, spines yellow to horn-coloured, straight  | up to 15 mm in size, spines white with a brown base, straight   | up to 8 mm in size, spines purely white, curved   |



Map 7: Present state of knowledge of the distribution area of *G. kuehhasii* and its subspecies *corneuspinum* and *incurvatispinum*.

## Cultivation

*Gymnocalycium kuehhasii* is somewhat sensitive to direct sunlight in cultivation, too, and prefers half shaded places. Heat is well tolerated under these conditions. When given plenty of water they grow strikingly fast (exception: subspecies *Incurvatispinum*) and are able to flower already after two to three years. Flowers appear two to four weeks earlier than with the other *Gymnocalycia* from the region north Córdoba (exception: *G. alenae*).

Table 3: Employed field numbers and synonyms as well as assignment to the species.

| <i>G. kuehhasii</i><br>subsp. <i>kuehhasii</i> | <i>G. kuehhasii</i><br>subsp. <i>corneuspinum</i> | <i>G. kuehhasii</i><br>subsp.<br><i>incurvatispinum</i> | <i>G. alenae</i> |
|--|---|---|------------------|
| KF93-128-472                                   | LB 4618   | MaW 10-226/357  | Tom 502/1        |
| JL 38  | LB 5562   | MaW 13-382/613  | MaW 10-237/370   |
| LB 5546  | LB 5563   | MaW 13-386/618  | SPE 376-178-18   |
| MaW 10-240/373                                 | MaW 10-248/382                                    | SPE 136-59-13   | TS 166           |
| MaW 13-380/611                                 | MM 1048   | SPE 142-63-13   | TS 1510          |
| SPE 131-57-13                                  | SPE 371-175-18                                    | TS 153  | TS 1397          |
| SPE 356-57-18                                  | SPE 373-176-18                                    | TS 470  | VoS 10-0795      |
| SPE 360-170-18                                 | Tom 12-733/1                                      | TS 660  | VoS 18-2347      |
| TS 169   | TS 178  | TS 665  |                  |
| TS 658   | TS 1517   | TS 976  |                  |
| TS 1407  | TS 1518   | TS 986  |                  |
| VoS 10-798                                     | VoS 10-807  | VoS 13-1380   |                  |
| VoS 13-1376                                    | VoS 18-2342                                       | VoS 13-1383   |                  |
| VoS 18-2327                                    | VoS 18-2344                                       |   |                  |

### List of employed acronyms

JL = Jacques Lambert

KF = Franz Kühhas

LB = Ludwig Bercht

MM = Massimo Meregalli

MaW = Mario Wick

SPE = Reiner Sperling

Tom = Tomáš Kulhánek

TS = Thomas Strub

VoS = Volker Schädlich

All photographs, if not stated otherwise, are from the authors.

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