Gymnocalycium mostii aggregate: Taxonomy in the northern part of its distribution area including newly described taxa

Radomír Řepka¹ and Petr Koutecký²

¹ Department of Forest Botany, Dendrology and Geobiocenology, Faculty of Forestry and Wood Technology, Mendel University, Zemědělská 3, CZ - 613 00 Brno, Czech Republic (e-mail: repka@menelu.cz);
² Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 31, CZ - 370 05 České Budějovice, Czech Republic (e-mail: kouta@prf.jcu.cz).

Summary: Quantitative and qualitative characters were collected from 11 natural populations of four taxa: Gymnocalycium prochazkianum, G. simile, G. simplex and G. bicolor. In this paper the results of one-way ANOVA of 11 quantitative characters using taxon identity as the single predictor, and results of PCA are published. Seven qualitative characters were evaluated out of a morphometric analysis.

The results show a mostly intermediary position of G. simile between G. prochazkianum and G. simplex, whereby G. simile is classified here as an intermediate type at subspecies level (likely to be of hybrid origin between the assumed parents G. prochazkianum and G. simplex, which grow close to each other). G. simplex, which did not have a valid name, is classified as a subspecies of Gymnocalycium prochazkianum for its morphological and geographical proximity, as well as its genome size, and is described here.

For the identification of the taxa also flow cytometry was used, which demonstrated diploid level and a high similarity of genome sizes of all the taxa. Notes on the morphological differences between G. bicolor Schütz nom. inval. and G. prochazkianum subsp. simplex are added.


Für die Identifikation der Taxa wurde auch Durchflusscytometrie vewendet, die für alle Taxa Diploidie und grosse Ähnlichkeit der Genomgrössen zeigte. Zusätzlich werden Angaben zu den morphologischen Unterschieden zwischen G. bicolor Schütz nom. inval. und Gymnocalycium prochazkianum subsp. simplex angefügt.

Introduction

Subgenus Scabrosemineum Demaio et al. of the genus Gymnocalycium includes several aggregates of related species with a certain variability which have taxonomically important seed characteristics in common (sensu Kreuzinger, 1935; Schütz, 1969; Metzing et al. 1995; Charles, 2009). This subgenus has ca. 1 mm long, moderately flattened oval seeds with an erect or rather oblique hilum-micropyle region with a more or less inacrostic edge (arillus), and all species show brown to dark brown or black testa with gibbose cells (except for Gymnocalycium castellanosii s.l.). This seed type is also found within the Gymnocalycium mostii group, however, Till & Amerhauser (2002) mention two subgroups differing in seed colour. Some authors also mention the purple colour of anthers and filaments as an important distinguishing character of these two branches (Till & Amerhauser 2002; Bercht, 2012). The entire group of related taxa around the basic species Gymnocalycium mostii s. str. includes, according to various authors, up to 8 taxa: Gymnocalycium mostii (Gürke) Britton & Rose (G. mostii s.str.), Gymnocalycium mostii f. kurtzianum (Gürke) H. Till & Amerhauser, Gymnocalycium mostii var. miradorense H. Till & Amerhauser, Gymnocalycium mostii subsp. valnicekianum (Jajó) Meregalli & Charles, Gymnocalycium bicolor Schütz nom.
indicated as transitional forms between two above has its own distribution area. In seed lists, plants = 22) prevail, but also tetraploids (2n = 44) are mentioned taxa (Lambrou & Till 1993, Till & useful taxonomic character and in case of assumed hybridization, it is necessary to check if the parental taxa and the assumed hybrid are of the same ploidy level.

In literature, remarks on the close relationship of G. prochazkianum and G. bicolor var. simplex can be found, and these two taxa are merged with G. gerseni as G. valnicekianum Jajó (Till & Amerhauser 2002) or as G. mostii subsp. valnicekianum (Charles 2009). The provisional name ‘G. bicolor’ for plants linked with G. prochazkianum and having their distribution area south and north of San Pedro Norte is inaccurate (see Till & Amerhauser 2002). In seed stock lists and in collections, these plants are also indicated as G. bicolor var. simplex or ‘northern bicolor’. These plants differ in morphology from G. bicolor (s.str.) and also from G. prochazkianum.

The latter taxon stands out by its very bizarre habit (Sorma 1999, Papsch 2002); the epidermis is covered with a grey bloom, the spines are mostly three in number, light-coloured and positioned in a T shape, the flowers have a short pericarpel and a conspicuously coloured throat, and the anthers are purple. A characteristic feature is the large beet-like storage root, which is conspicuously constricted under the hypocotyl. G. bicolor var. simplex is a taxon resembling G. bicolor (s. str.), which was described by Schütz (1962) from the surroundings of the town Cruz del Eje. It has similar spine colour, however, it differs with flower architecture, other differential details and has its own distribution area. In seed lists, plants indicated as transitional forms between two above mentioned taxa (G. prochazkianum and G. bicolor var. simplex) are also offered.

In the subgenus Scabrosemineum, diploids (2n = 22) prevail, but also tetraploids (2n = 44) are known to occur there (Lambrou & Till 1993, Till & Lambrou 1998). Ploidy level can therefore be a useful taxonomic character and in case of assumed hybridization, it is necessary to check if the parental taxa and the assumed hybrid are of the same ploidy level.

All these circumstances motivated us to make a field study of populations of the G. mostii aggregate from the northern part of its distribution area. The aim of this paper is to summarize morphological similarities and differences between the taxa, to present data on ploidy levels / genome sizes, and to clarify nomenclature of some taxa of the group.

Material and methods
Collection and morphological data evaluation
During three field trips in Argentina (2010-2012) morphometric data were collected from natural populations of 4 taxa: G. bicolor (3 populations), G. simplex (4 populations), G. prochazkianum (2 micropopulations of one population) and populations morphologically intermediate between the last two taxa, G. simile (3 populations) at selected locations in the northern part of the province of Córdoba. The populations of G. simplex were collected at locations distant from G. prochazkianum and G. simile populations to avoid possible products of a gene flow. From each population, 30 mature plants (in order to have generative organs) were randomly selected. On these, the following quantitative characters were measured: Body diameter, number of ribs, areole distance, number of spines, spine length, flower length, fruit length & width, and seed size (seed length, seed width and their ratio). For seed characters, 50 randomly selected seeds collected from natural populations were measured.

The quantitative morphological data were analysed with one-way ANOVA using taxon identity as the single predictor. Values of some characters were log-transformed prior to analysis to improve the distribution and homogeneity of variance (Tables 1 & 3). Two analyses were performed, one focused on G. prochazkianum, G. simile and G. simplex, and the other on G. bicolor and G. simplex. Differences between the taxa in the former analysis were analysed using the Tukey HSD test for unequal numbers of samples. The analyses were computed using the Statistica 8 software.

Principal component analysis (PCA) based on a correlation matrix was computed for G. prochazkianum, G. simile and G. simplex. Five variables, for which data were available for most of the individuals, were used (body size, number of ribs, areole distance, number of spines, and spine length). The analysis was computed using Canoco for Windows 4.5 (ter Braak & Šmilauer 2002).

All measured and evaluated characters were used for both taxon descriptions and comments. The distribution map of the compared taxa was compiled based on our own field data and on data
of the distribution of the taxa available on the internet (incl. M. Meregalli and M. Tvrdík – pers. comm.).

Studied populations (** samples used for flow cytometry):

**G. prochazkianum** subsp. **prochazkianum** (referred to as **G. prochazkianum** in the text)
1. Quilino, 3.78 km SE of the village**
2. Quilino, 4.30 km SE of the village

3. **G. prochazkianum** subsp. **simplex** (referred to as **G. simplex** in the text)
1. San Pedro Norte, 2 km ENE of the church in the town
2. San Pedro Norte, 3.78 km NNE of the church in the town**
3. San Pedro Norte, S of Chuña Huasi, 10.9 km NNE – N of the town
4. Caspi Yacu, 1.75 SSW of the settlement

Seeds of this taxon were also measured from these populations:
5. Los Algarrobos, 0.6 km E of the hamlet, by small road to San Pedro Norte
6. Chuña Huasi, 1 km S of the settlement
7. Totorillas, 0.4 km E of the village
8. Pozo Nuevo -Pozo del Tigre, 2.25 km WNW of El Puestito

**G. prochazkianum** subsp. **simile** (referred to as **G. simile** in the text)
1. Orcosuni, Cerro Horqueta, 0.4 km N of the village**
2. Villa Quilino, Los Algarrobos, hillocks 2.15 km NE of the hamlet
3. Orcosuni, Cerro Banado, 2.0 km ENE of the settlement

**G. bicolor** nom. inval. (referred to as **G. bicolor** in the text)
1. Cruz del Eje, SW tip of the Dique Cruz del Eje dam
2. Canteras Quilpo, 4.7 km NNW of the village
3. Canteras Quilpo, 3.1 km SE of the village

Flow cytometry

Genome size and DNA ploidy level were determined using a Partec CyFlow SL flow cytometer (Partec GmbH., Münster, Germany) with a 532 nm (green) diode-pumped solid-state laser (100 mW output). The samples were prepared following the simplified two-step protocol of Doležel et al. (2007). A (two to three month old) seedling was chopped together with about 5 mm$^2$ of the internal standard using a sharp razor blade in a Petri dish containing 0.5 ml of ice-cold Otto I buffer (0.1M citric acid, 0.5% Tween-20). The suspension was filtered through a 42-μm nylon mesh and incubated for at least 10 min at room temperature. After incubation, 1 ml of the staining solution was added. The staining solution consisted of Otto II buffer (0.4M Na$_2$HPO$_4$,12H$_2$O), 2-mercaptoethanol (2 μl/ml), RNase IIa (50 μg/ml) and propidium iodide (50 μg/ml). Samples were run on

<table>
<thead>
<tr>
<th>Character</th>
<th>Mean</th>
<th>transf</th>
<th>df</th>
<th>F</th>
<th>p</th>
<th>R$^2$</th>
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<tbody>
<tr>
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<td>40.26</td>
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<tr>
<td>Number of ribs</td>
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<td>13.8</td>
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<td>189.40</td>
</tr>
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<td>17.1</td>
<td>log</td>
<td>2,237</td>
<td>39.21</td>
</tr>
<tr>
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<td>8.2</td>
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<td>250.00</td>
<td>2.2E-16</td>
</tr>
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<td>88.81</td>
<td>2.2E-16</td>
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<td>9.89</td>
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<td>11.8</td>
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<td>3.43</td>
<td>0.035</td>
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<td>1.55</td>
<td></td>
<td>2,165</td>
<td>8.69</td>
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<td>1.00</td>
<td>0.93</td>
<td>log</td>
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<td>32.69</td>
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<td>Seed width</td>
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<td>0.70</td>
<td>0.66</td>
<td>log</td>
<td>2,595</td>
<td>56.91</td>
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<tr>
<td>Seed length/width</td>
<td>1.34</td>
<td>1.43</td>
<td>1.42</td>
<td>log</td>
<td>2,595</td>
<td>24.77</td>
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</tbody>
</table>

Table 1 Analysis of variance testing the morphological differences between **G. prochazkianum**, **G. simile** and **G. simplex** (taxon identity was used as the only predictor in one-way ANOVA). Abbreviations: transf – transformation applied, df – degrees of freedom, F – value of F-statistics, p – significance level, R$^2$ – coefficient of determination. Results of Tukey HSD tests testing differences between individual taxon means are included in Figure 1.
the flow cytometer after several minutes of staining and a fluorescence intensity of 5000 particles was recorded. The fluorescence histograms were analysed using the FloMax 2.60 (Partec GmbH.) and FlowJo 7.6.5 (TreeStar Inc.) software. *Pisum sativum* L. ‘Ctirad’ (2C = 9.09 pg, Doležel et al. 1998) was used as the primary reference standard. However, due to the high degree of endopolyploidy in Cactaceae and overlap of some peaks with the primary standard, we used *Bellis perennis* L. calibrated against the primary standard as the internal standard (2C = 3.62 pg).

**Results and Discussion**

**Quantitative characters**

Quantitative morphological characters of *G. prochazkianum*, *G. simile* and *G. simplex* are summarized in Table 1 and Figures 1 & 2. Table 1 shows the results of the analysis of variance. The taxa compared are mutually different in all tested quantitative characters. Values of *G. simile* are generally intermediate between the values of other two taxa, *G. prochazkianum* and *G. simplex*, which suggests its possible hybrid origin. The intermediate position of *G. simile* is particularly shown by body diameter, number of ribs, areole distance, number of spines, spine length, fruit length, and fruit length/width ratio (see Figure 1).

Table 2 shows the average variability of the morphological characters measured in all three taxa (*G. prochazkianum*, *G. simile*, and *G. simplex*). The highest variability is shown by spine length, body diameter, fruit shape and number of ribs in *G. prochazkianum*, by number of spines and fruit width in *G. simile*, and by fruit length and areole distance in *G. simplex*. The highest character variability as a whole was surprisingly found in *G. prochazkianum* and not, as could have been expected, in possibly hybridogenous *G. simile*.

**Figure 1** Box-and-whisker plots of the morphological characters of *G. prochazkianum*, *G. simile* and *G. simplex*. Median, quartiles, non-outlier ranges and outliers are depicted by a bold line, boxes, whiskers and points, respectively. Letters above the graphs indicate a statistically significant (p < 0.05) difference inferred from the post-hoc multiple comparisons using Tukey HSD tests applied to the ANOVA results (Table 1). Note that for the fruit characters the significance tests are highly influenced by a very low number of observations for *G. prochazkianum*.
Box-and-whisker plots of the morphological characters of seeds of *G. prochazkianum*, *G. simile* and *G. simplex*. Median, quartiles, non-outlier ranges and outliers are depicted by a bold line, boxes, whiskers and points, respectively. Letters above the graphs indicate a statistically significant (p < 0.05) difference inferred from the post-hoc multiple comparisons using Tukey HSD tests applied to the ANOVA results (Table 1).

### Table 2

<table>
<thead>
<tr>
<th>Morphological character / taxon</th>
<th><em>G. prochazkianum</em></th>
<th><em>G. simile</em></th>
<th><em>G. simplex</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body diameter</td>
<td>33.5</td>
<td>23.3</td>
<td>24.9</td>
</tr>
<tr>
<td>Number of ribs</td>
<td>15.6</td>
<td>15.7</td>
<td>17</td>
</tr>
<tr>
<td>Areoles distance</td>
<td>22.3</td>
<td>17.7</td>
<td>20.7</td>
</tr>
<tr>
<td>Number of spines</td>
<td>31.6</td>
<td>29.4</td>
<td>22.3</td>
</tr>
<tr>
<td>Spine length</td>
<td>49.1</td>
<td>37.1</td>
<td>26.2</td>
</tr>
<tr>
<td>Fruit length</td>
<td>15.4</td>
<td>21.3</td>
<td>18.8</td>
</tr>
<tr>
<td>Fruit width</td>
<td>11.8</td>
<td>15.8</td>
<td>15.8</td>
</tr>
<tr>
<td>Fruit length/width ratio</td>
<td>19.9</td>
<td>15.7</td>
<td>14.5</td>
</tr>
<tr>
<td>Seed length</td>
<td>8.3</td>
<td>7.9</td>
<td>10.4</td>
</tr>
<tr>
<td>Seed width</td>
<td>10.4</td>
<td>10.8</td>
<td>9.6</td>
</tr>
<tr>
<td>Seed length/width ratio</td>
<td>9.7</td>
<td>9.2</td>
<td>9.6</td>
</tr>
</tbody>
</table>

### Table 2 Coefficient of variability (%) of morphological characters for 3 taxa, *G. prochazkianum*, *G. simile* and *G. simplex*.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>n</th>
<th>DNA ploidy level</th>
<th>Mean 2C-value</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. prochazkianum</em></td>
<td>3</td>
<td>2x</td>
<td>3.92</td>
<td>0.01</td>
</tr>
<tr>
<td><em>G. simile</em></td>
<td>3</td>
<td>2x</td>
<td>3.97</td>
<td>0.01</td>
</tr>
<tr>
<td><em>G. simplex</em></td>
<td>3</td>
<td>2x</td>
<td>3.97</td>
<td>0.02</td>
</tr>
</tbody>
</table>

### Table 3

Genome sizes of *G. prochazkianum*, *G. simile* and *G. simplex* (n – number of studied individuals; mean 2C values and their standard errors (SE) are indicated in pg of DNA).
PCA

Only 5 untransformed quantitative characters were included into the analysis. A total of 240 plants were analysed, i.e. 30 plants from 8 populations of all three taxa compared.

There is a clear morphological separation of *G. prochazkianum* and *G. simplex* mainly along the first ordination axis. *G. simile* has an intermediate position between the other two taxa (Figure 3), but it overlaps more with *G. prochazkianum* than *G. simplex*. The first and second ordination axes are depicted, which explain 66.6 % and 14.9 % of the variation, respectively.

By means of projection of the variables we can describe the relations between characters and their weight, i.e. how much the characters contribute to the variability of each depicted component. All characters have similar weight (slightly smaller for body diameter and spine length). The number of spines and number of ribs are strongly correlated.

Qualitative characters

Roots. In *G. prochazkianum* the root has a very characteristic shape, which is markedly different from that of *G. simplex*: it is a beet-like storage root, single or sometimes branched, conspicuously constricted under the hypocotyl. The root of *G. simile* is in some cases similar to *G. prochazkianum*, i.e. robust, beet-like, but mostly without a conspicuous constriction under the...
hypocotyl; in plants that are morphologically closer to \textit{G. simplex}, roots are also more similar to this taxon. The roots of \textit{G. simplex} are rope-like, however, often slightly thickened under the hypocotyl, growing vertically and spreading, branched.

Body shape. The body may be at most short columnar in \textit{G. simplex}, otherwise it is flat spherical to hemispherical, in young specimens up to ½ embedded in the soil, in older ones completely emerged above the ground. The body of \textit{G. prochazkianum} is embedded in the soil for up to 3/4, in older specimens flattened hemispherical. \textit{G. simile} is variable in this character and similar to both subspecies, more often embedded in the soil, some plants are completely emerged above the ground (the frequency varies strongly for each population) (Figure 4).

Epidermis bloom. The epidermis bloom (patina) of \textit{G. prochazkianum} is very conspicuously developed, having the appearance of cement powder, but it is very variable in young, middle-aged and very old plants and it also varies in plants growing in the shade or in full sun. This cover is undoubtedly caused by the extreme ecological conditions of the habitat. When young, the epidermis is rather brown, and then it gradually becomes brownish grey-green. In mature plants the bloom is ± markedly developed, by which the plants become hardly noticeable between the coarse light-coloured rubble of porphyric microgranite (mimicry). In old and shaded plants the bloom is rather inconspicuous, and the epidermis has no bloom and is intensively green-grey. \textit{G. simile} has a conspicuous grey-green to green-grey epidermis, and a bloom appears only in some plants, especially young plants more similar to \textit{G. prochazkianum}. In old plants it is rare and never as conspicuous and extensive as in \textit{G. prochazkianum}. Plants of \textit{G. simplex} never have such a bloom. In this character \textit{G. simile} thus takes an intermediate position.

Spine diameter. The spines in \textit{G. prochazkianum} are lengthwise sulcate, especially the spine pointing downwards, but all spines have roughly the same rounded triangular cross-section. Their diameter is mostly 0.7–0.9(-1.1) mm; their base is not bulbously thickened. In \textit{G. simile} the spines are very different in cross-section: they are flat and also short, angular just as in \textit{G. prochazkianum}, circular in cross-section and longer just as in the spines at the upper edge in \textit{G. simplex}, or show a combination of these spine types. Their diameter is also variable, (0.8–)1–1.3 mm. The spines are markedly bulbously thickened at the base. \textit{G. simplex} has its spines at the upper margin spherical or oval just as the central ones, but the three lower ones are angular or flattened. Especially the one pointing downwards is typically ensiform (flattened) and sometimes slightly curved upwards. All spines are markedly bulbously thickened at the base and are mostly 0.9–(1.0–1.1) mm in diameter.

Spine colour. \textit{G. prochazkianum} has conspicuous spines for their pinkish, later light grey to whitish colour. \textit{G. simplex} has typically bicoloured marginal spines, i.e. the lower three are bluish grey just as the central ones and the other are whitish or ivory-coloured. \textit{G. simile} often has whitish spines when young, but later some similarity to \textit{G. simplex} become apparent: the spines become often bicoloured (i.e. the lower three marginal spines turning grey-blue) or show still other combinations intermediate between other two subspecies.

Flowers. The flowers are similar in all three taxa: they have a markedly short pericarpel (although longer than the perianth when including the receptaculum) and a short perianth, which has an ivory or light pink colour. \textit{G. simile} most often has an ivory colour. The flower throat is purple to purplish-violet in all investigated taxa, but colour intensity varies and is strongest in \textit{G. simile} and \textit{G. simplex} (see Figure 5). The flowers of \textit{G. prochazkianum} are the most often pinkish of the three taxa, but in contrast to the other two taxa they do not have such marked violet-pink central stripes on the tepals. The colour of filaments, the arrangement of anthers in the flower as well as the colour of anthers are identical in all three taxa. The filaments are violet-pink at least in their lower half, being the least intense in \textit{G. prochazkianum}, and the anthers are markedly violet-pink before the flower opens (Figure 5). The shape of the scales covering the pericarpel and receptaculum is close to oval in \textit{G. simplex}, but less than hemispherical in the other two taxa. The size of flowers in nature and in culture sometimes strongly differs (see Figure 5, \textit{G. simplex}, C-D). To complete the survey, \textit{G. mostii} s. str. plants have flowers of up to 7–8 cm long (see Figure 5F).

Seeds. All three taxa have oval to oval-oblong seeds, which are mostly slightly incurved and possess a testa covered with shiny hemispherical warts. Their shape is characterised by their length/width ratio. As shown in Figure 6, there is some variation in the studied populations. \textit{G. prochazkianum} has the most oval seeds whereas the two other taxa have more elongated and lengthwise incurved seeds. In this qualitative character (seed shape) \textit{G. simile} and \textit{G. simplex} are nearly identical.

Seed width differs between the three taxa. The colour of unripe or near-ripe seeds is reddish, whereas fully ripe ones have the colour of the mineral almandine or Bohemian garnet, exceptionally
even darker. The opinion that one of the basic differences between the branches (species) of the *G. mostii* agg. is the seed colour, is completely erroneous (cf. Till & Amerhauser 2002: 450). The conclusion of an examination of seeds of all members of the *G. mostii* aggregate is that the seeds of all taxa are nearly identical in testa colour as well as their shape. Differences found in the testa colour had only been caused by the degree of seed ripeness. In one or two branches of the aggregate
the testa may be red or black-red, but the seeds of all aggregate members are very similar in shape. In all studied populations the shape (length/width ratio) and also length and width of the seeds varied, however the variability was low. The variation coefficient is mostly 7-10%, only the variation of seed width in population G. simplex no. 8 amounts to 12.4%. The seeds of G. prochazkianum are (in contrast to G. simile and G. simplex) more oval, lengthwise not much incurved, dark (up to red-black), the hilum shorted and broader, less angular, and positioned more perpendicularly on the seed end (Figure 6).

Flow cytometry

Both polyploidy and genome size differences are known in the genus Gymnocalycium and might provide a breeding barrier: diploid, tetraploid and hexaploid taxa are reported (Lambrou & Till 1993; Till & Lambrou 1998) and genome size variation within a ploidy level is up to 16% (Repeka & Koutecky, unpublished data). G. prochazkianum, G. simplex and G. simile are all DNA diploids and their genome sizes are nearly identical (Table 3). The difference between the G. prochazkianum and G. simplex is ca. 1.2%, which is below the measurement error of flow cytometry. These data confirm that there are probably no
barriers to possible hybridisation caused by different ploidy levels or large differences in the genome size.

Eco-geographical notes

Figure 7 shows the distribution areas of G. prochazkianum, G. simile and G. simplex. The areas are not connected but isolated from each other by belts in which none of the species occurs. G. prochazkianum has the southernmost situated distribution area of all three taxa. It basically consists of one large plant population (one site) situated in the hillocks SE of the town of Quilino, along the R 60 road (on its north side). Nevertheless, the entire population consists of several small micropopulations isolated from each other by human activities, but also by ecological specialisation of this species. On slopes and tops of the low flat hills, the species grow mostly in groups, but at the foot it is missing or rare. G. prochazkianum colonises stony clearings of the Chaco árido shrub formation (sensu Cabrera 1976) with Acacia furcatispina and A. caven as dominant species. It mostly avoids the shrub cover, but single plants can also be found under the shrubs. The prevailing rock type is biotitic fine-grained granite pervaded with younger granitic derivates, particularly hard porphyric microgranites with automorphous silica phenocrysts, to a lesser extent alkaline feldspars are also included. This hard rock disintegrates here into a skeleton with prevailing fractions of 5-30 cm, mixed with loam. G. prochazkianum is very well adapted to these extreme, strongly desiccating and very nutrient-poor substrates: single plant bodies are immerged into the substrate for up to 2/5–3/4 and have a very conspicuous beet-like storage root that penetrates deep into the stone rubble. The permanent character of a grey bloom on its epidermis is caused by the movement of hot air from the sun-heated depressions in the surrounding of the site to the low hillock around Quilino. Through this hue G. prochazkianum plants have acquired mimicry in the disintegrated porphyric microgranite. The question whether the G. prochazkianum population is (1) a primarily small one or (2) a relic of an originally larger one still remains unanswered. However, regarding the narrow ecological specialisation to a certain community growing on a distinct type of bedrock, whereby this ecological situation is not found anywhere else in the surrounding, the first option appears to be the most probable.

G. simplex has a relatively large distribution area situated to the north of the G. prochazkianum area, beginning in the uplands between the hamlet of Los Algarrobonos and the town of San Pedro Norte (Figure 7). It continues, however, in a relatively wide zone through the low and flat Sierra de Ambargasta range up to the boundaries of the provinces Córdoba and Santiago del Estero. According to present knowledge its occurrence ends here, however, the presence of similar habitats across the boundary indicate that it might also be found more to the north. This taxon is associated with crumbly granite outcrops, but it also grows on sandy granite crumbles, exceptionally on much harder rock (rhyolitic porphyry or ferruginous quartz granite with transitions to granitegneiss) in the northern part of its distribution area. It is a plant of rock fissures and rock edges even though it does not seem to be equipped for these habitats. It has no round (storage) root; the roots are rope-like and at most slightly thickened in the upper part under the hypocotyl. G. simplex excellently resists high temperatures and drought in these sun-exposed habitats and mostly avoids shrub cover, even though its entire body is situated above the ground and its epidermis is dark green and does not have the protective grey bloom of G. prochazkianum. It grows, similarly to G. prochazkianum, on soils poor in nutrients.

The third taxon, G. simile, grows at several locations between the distribution areas of its supposed parental species (taxa), but is now sufficiently isolated and not connected with either of them. It has so far been found at three relatively proximate localities (Los Algarrobonos, Cerro Horqueta, Cerro Banado) in rather numerous populations which are associated with open shrub formations of stony slopes with or without Acacia furcatispina, but commonly with A. caven and other shrub species. The geological conditions are similar to the locality of G. prochazkianum. The rock is granitic and includes frequent transitions to more coarse-grained variants with occasional occurrence of pegmatic textures, but porphyric microgranites are missing. It grows exceptionally also on gravelly-sandy granite crumbles, there with ecologically more resembling G. simplex, but in all cases again outside of shrub cover. All three populations are strongly variable in all characters. Plants can be found here with characters varying between those of its supposed parental species, and entire groups of plants can locally be considered good intermediate forms. Two important characters, the epidermis bloom and the spherical beet-like root, and in connection with these also other morphological characters, vary from plant to plant. All plants produce fruits with partially or fully germative seeds and form independent populations of plants in all development stages. In some years the seeds ripen imperfectly, have low germination and are more reddish than in the years in which they fully ripen (see Figure 6 D & F).
**Threats**

*G. prochazkianum* at its single location SE of Quilino is to be considered a critically endangered species, growing in several micropopulations including just a few hundred plants. Most plants are hidden from collectors’ eyes between stones or also under shrubs, and thanks to the very hardly penetrable vegetation with *Acacia furcatispina* it seems to be protected so far. Grazing, if taking place here, only concerns the marginal part of the species distribution area. The greatest risk here is collectors, who have reduced a considerable part of the population since the species was discovered by digging out plants and transporting them out of Argentina.

*G. simile* grows in relatively large populations at the three locations known to date. One of them has often been visited by gymno-enthusiasts lately. As far as we have observed, no plants have been dug out; the plants produce enough seeds, which satisfies visitors of the location sufficiently.

*G. simplex* is a taxon spread over many locations of its relatively extensive distribution area, where populations comprise dozens or rather hundreds to thousands of plants. It can therefore be considered a common taxon and is not threatened.

**Descriptions**

Based on the results of analyses of quantitative and qualitative morphological characters and genome size, we regard all three taxa to be subspecies of one species, *G. prochazkianum*, a separate species belonging to *G. mostii* agg.

*Gymnocactus prochazkianum* subsp. *simplex* Řepka subsp. nov.

Stem flat to flattened globose, exceptionally elongate, (36-)50-111(-250) mm in diameter and 30-80(-130) mm tall. Roots medium long, branched, rope-like or sporadically thickened or branched under the hypocotyl. Epidermis matt, green or light purplish green. Ribs (9-)10-18(-21), pectinately arranged, upper pair short, the adjacent one or two pairs the longest, the three lower ribs shorter than the former, whitish to ivory-coloured, round or angular in cross-section, lower 1-3 ribs bluish grey, the lower one pointing downward, straight or slightly curved, flattened rhombic(-ensiform) in cross-section, longitudinally sulcate. All spines or only the lower radial and central ones bulbously thickened at base, more often slightly standing out, rarely appressed to the body, (6-)10-24(-30) mm long; colour contrast between upper and lower radial spines clearly visible already when juvenile, even without moistening. Central spines (0-)1-4(-5), missing in young plants, straight in older ones, round in cross-section, subulate, rigid, grey-blue.

Flowers bisexual, short funnel-shaped, 40-45(-50) mm long and 35-40 mm wide; in culture up to 60 mm long and 50 mm wide, perianth much longer than pericarpel incl. receptaculum; pericarpel very short, 8-12 mm long and 8-10 mm wide, dark or grass-green, shiny, without pruinose bloom. Outer perianth segments oblong to spathulate to scapulate, pale pinkish whitish, with a broad greenish brownish stripe on the outside, at base and margin pale purplish, fluently passing into the scales of the pericarpel. Inner perianth segments oblanceolate to oblong, apex with tip, tapering to the base, whitish rose to pale pink, with a darker central stripe, at base often pale purplish or pale rose, 19-30 mm long and 4-5 mm wide. Receptaculum funnel-shaped, 10-15 mm high, inside deep purple. Style whitish to colourless, purplish at base, penetrating the bottom of the receptaculum, 10 mm long; stigma slightly yellowish, 9-10-purple. Stamens growing in several rings, the lower shorter than the style and rising from its base, the next ring rising from the receptaculum bottom, above them another 2-3 rings, all at least in their lower half attached to the receptaculum wall; their lower half purple, the upper half whitish; except for the small lower ring the anthers of all other stamens surpassing the top of the stigma. Anthers purplish before opening, after opening pale yellowish horny. Ovary obovate to conic, 6-12 mm high and 5-8 mm wide, cavity whitish, wall up to 1.5 mm thick. Fruit ovoid to slightly pyriform, light or grass-green, up to pale green when fully mature, matt, (11-)14-25(-30) mm long and (8-)9-15(-16) mm wide; scales 5-7, near-hemispherical, obtuse, whitish and green at the base.

Seeds ovate to oblong, longitudinally slightly incurred, laterally slightly flattened, (0.7-)0.8-1.1(-1.2) mm long and (0.5-)0.55-0.8 mm wide (subgenus *Scabrosemineum*). Testa covered with convex hemispherical cells, red-brown (colour of the mineral almandine) to red-black, matt to faintly shiny. Hilum-micropyle region obliquely perched, elongated to broadly elongated, its margin slightly curved outwards, straight or slightly convex, faintly embedded, whitish, in the lower part with a minute round micropyle.

Type: Argentina, Córdoba Province, Sobremonde Department, village of Chuña Huasi, pas-
Figure 5A Flowers of *G. prochazkianum* 2

Figure 5B Flowers of *G. simile* 1

Figure 5C Flowers of *G. simplex* 1

Figure 5D Flowers of a cultivated *G. simplex*.

Figure 5E Flowers of *G. bicolor* Piltz 116 from Cruz del Eje

Figure 5F Flowers of *G. mostii* “type” (sensu B. Schütz, ex coll. Schütz, Brno).

Figure 5G Flowers of *G. mostii* from Carlos Paz.

Figure 5H Flowers of *G. genseri* nom. nud. *Piltz* 380 from Sauce Punco
Figure 6A Seeds of *G. prochazkianum* 1

Figure 6B Seeds of *G. prochazkianum* 2

Figure 6C Seeds of *G. prochazkianum* STO 1581

Figure 6D Seeds of *G. simile* 1

Figure 6E Seeds of *G. simile* 2

Figure 6F Seeds of *G. simile* 1 (the seeds collected in another year, cf. Figure 6D)

Figure 6G Seeds of *G. simplex* 8

Figure 6H Seeds of *G. simplex* 7
ture with rocky patches on small hill along road ca 3 km south of the village, 890 m above sea level, 26 November 2012, coll. Radomír Řepka (no. RER 707), CORD (holotype).

*Gymnocalycium prochazkianum* subsp. *simile* Řepka, subsp. nov.

Stem flat to flattened globose, when young similar in habit to *G. prochazkianum*, when old with an intermediate habit (41-)-50-110(-122) mm in diameter and 10-50 mm tall. Roots long, rope-like, branched, more often however globose to beet-like. Epidermis matt, grey-green to brown-green, often covered with a faint greyish bloom, often with a light purplish (or pinkish) hue, rarely dark green. Ribs 8-14(-15), straight, in young plants flat, in mature plants more or less markedly gibbous with undulate notches between them, divided by smaller, shallow cross notches into large inflated and rounded bumps, in the lower half narrowed and under the areoles elongated with distinctive rounded or (slightly) angular warts on top; in lower part 10-18 mm, in upper part 8-10 mm broad. Areola short oval, not embedded, but slightly embedded when old, when young covered with copious whitish greyish wool, later disappearing or discolouring to dark grey, at a distance of (12-)-14-24(-28) mm from each other.

![Distribution map of the three studied *Gymnocalycium* taxa (*G. prochazkianum*, *G. simile*, and *G. simplex*).](image)
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Table 4 Analysis of variance testing the morphological differences between *G. bicolor* and *G. simplex* (taxon identity was used as the only predictor in one-way ANOVA). Abbreviations: transf – transformation applied, df – degrees of freedom, F – value of F-statistics, p – significance level (n.s. – not significant), R² – coefficient of determination.

**Figure 8** Box-and-whisker plots of the morphological characters of *G. bicolor* and *G. simplex*. Median, quartiles, non-outlier ranges and outliers are depicted by a bold line, boxes, whiskers and points, respectively. The taxa significantly differ in all characters except for number of ribs, spine length and fruit length/width ratio (Table 4).
Radial spines 3-7(-9), pointing to the side, the lower odd one pointing downwards straight or sometimes slightly curved upwards if there are three arranged in a T shape, but if there are five, the upper pair is shorter, its spines are ± rounded and whitish in cross-section, and the other pair or the lower three are flattened, longitudinally sulcate (or sometimes rounded in cross-section), whitish ivory to chalk white, rarely pinkish or grey-blue. Exceptionally all radial spines flattened and bluntly or sharper angular, and all of them (5-7) grey-blue. Only in a few plants all spines thickened at base, rather often slightly standing out on the body, (5-7)22(-28) mm long, in some plants markedly robust (1.0-2.5 mm broad or in diameter). Central spines 0-2, missing in young plants, in older plants missing or one, rarely two developed, straight or slightly curved, pointing straight upwards, rounded in cross-section, subulate, rigid, whitish ivory.

Flowers bisexual, short funnel-shaped, 40-48(-55) mm long and 30-35 mm wide, perianth longer than pericarpel (incl. receptaculum); pericarpel short, 12-16 mm long and 5-7(-12) wide, dark green or greyish green, matt, with light pruinose bloom or dark brow-pink hue. Outer perianth segments oblong to slightly spathulate, brown-green with narrow whitish pinkish margins, at base purplish, fluorently passing into hemispherical pericarpel scales. Inner perianth segments oblanceolate, apex with tip, tapering to the base, pale whitish rose, with a pink-brownish central stripe, at base purplish, 15-20 mm long and 4-7 mm wide. Receptaculum funnel-shaped, 10-12(-15) mm high, inside deep purple. Diaphragm between nectar chamber and ovary colourless. Style whitish, sometimes slightly purplish at base or entirely, 6-10 mm long; stigma slightly yellowish, 8-12 armed. Stamens growing in several rings, the lower one shorter than the style, rising from the receptaculum wall 3-4 mm above the style base, next ring rising from the stigma base with continuously other rings above it. All are attached to the receptaculum wall for at least their lower half, which is purple, whereas the upper half is whitish; except for the lower stamens all surpassing the top of the stigma. Anthers purple to purplish violet, after opening covered by a yellow colour. Ovary short ovate to elongated, sometimes narrowed at base, 10-16 mm high and 8-12 mm wide, cavity whitish, wall 2.0 mm thick. Fruit ovoid, sometimes up to oblong-ovate, grey-green, matt, (14-)15-28(-30) mm long and (9-)10-16(-18) mm wide; scales 5-7, near-hemispherical, obtuse, whitish pinkish and grey-green at the base. Seeds ovoid-oblong, longitudinally incurved, laterally slightly flattened, (0.8-)0.9-1.2(-1.3) mm long and (0.55-)0.6-0.8(-0.9) mm wide (subgenus Scabrosemineum). Testa covered with hemispherically convex cells, conspicuously red to dark red, exceptionally up to red-brown (colour of the mineral almandine), matt to faintly shiny. Hilum-micropyle region obliquely perched to deflected as seen from the longitudinal axis of the seed or

Figure 9 Box-and-whisker plots of the morphological characters of seeds of *G. bicolor* and *G. simplex*. Median, quartiles, non-outlier ranges and outliers are depicted by a bold line, boxes, whiskers and points, respectively. The taxa significantly differ in all three characters (Table 4).
partly angular, oblong, whitish, in its lower part with a minute rounded micropyle.

Type: Argentina, Córdoba Province, Tulumba Department, village of Orcosuni, between shrubs on N slope of Cerro Horqueta, 0.4 km N of the village, c. 550 m above sea level, 25 November 2012, coll. Radomír Řepka (no. RER 701), CORD (holotype).

In *G. simile* populations three morphologically different types can be distinguished, which approximate one of both marginal subspecies in characters or have intermediate characters (Figure 4).

Notes on origin of *G. simile*

*G. simile* is morphologically intermediate between other two related taxa (*G. prochazkianum* subsp. *prochazkianum* and *G. prochazkianum* subsp. *simplex*), as we have shown here. It is probably of hybrid origin (as indicated by the studied morphological characters, genome size, ecology and distribution). For final confirmation of the hybrid origin, however, further research and the use of molecular methods is required. As a possible alternative hypothesis (less likely) is the origin of all three taxa as derivatives of one common ancestor. These derivates could have developed in spatial isolation and partly different environmental conditions, and thus adopted different morphology.

Comparison of *G. bicolor* and *G. simplex*

In the analysis of variance confirmed morphological differentiation of *G. bicolor* and *G. simplex*, eight out of eleven morphological characters (including seeds) turned out to be significant (Table 4, Figures 8 & 9). The tests demonstrated very significant differences of these taxa in body diameter, number of spines, and seed length and width, and smaller (albeit still statistically significant) differences in areole distance and seed length/width ratio. At the same time, however, the tests did not prove differences in number of ribs, spine length and fruit length/width ratio.

Flower characters

1. Flowers of *G. bicolor* are in most cases longer and wider than those in *G. simplex* (Figure 5), but this character can be influenced by ecology of the habitat, weather conditions and culture.

2. The ratio pericarpel + receptaculum to perianth in *G. bicolor* is 0.8-1.0, in *G. simplex* it is 0.7-1.7.

3. The perianth colour in *G. bicolor* is white, whereas in *G. simplex* it is faintly pink with a conspicuous central purple stripe, which is deeper coloured in the lower half.

4. The throat (= receptaculum) colour in *G. bicolor* is pink to faintly pink, in *G. simplex* it is purple to violet-purple.

5. In *G. bicolor*, the scales on the pericarpel and outer perianth segments have the same colour as the perianth, the lower green ones have no clear margin or a whitish pinkish one; in *G. simplex* green-greyish to dark green, rigid scales with a pink-purple margin dominate.

6. The scales and leaves of the outer perianth have dark (purple) spots at their tops in *G. bicolor*, which are absent in *G. simplex*.

7. The stamens in *G. bicolor* are pink in their lower part, whitish in the upper part, whereas in *G. simplex* they are violet-purple, sometimes also in the upper part.

8. The anthers in *G. bicolor* are greyish yellowish before opening, in *G. simplex* they are purple before opening, yellow after opening.

Spines

The arrangement of the spines is similar in both species, however, there are certain differences in cross-section, colour and other characters.

1. *G. bicolor* has all spines round to oval, sometimes even quadrangular in cross-section, the three lower radial spines are grey after moistening, (pink-reddish when dry), the lower spine pointing towards the ground is one of the shortest in the areole, the others are longer, straight, ivory-coloured with a brown or black tip, the number of radial spines is usually higher than in *G. simplex*. Both taxa have 1-4 central spines, mostly coloured differently from the other spines.

2. *G. bicolor* has thinner spines, (0.7-)0.8-1.0 mm in diameter (measured in the middle part of the spine); *G. simplex* has spines which are round, flattened or rounded angular in cross-section, without a conspicuous colouring at the tip, the lower three radial spines are grey, the lower spine is flat to flattened and often slightly curved upwards. They are thicker, mostly 0.9-1.0-1.1 mm in diameter. Spines in both species are bulbously thickened at the base.

The ecology of both species is similar. They are both species of rock fissures, stony or sandy-stony, desiccating and nutrient-poor substrates. Both have a green epidermis and rope-like roots. However, each one grows in its own distinct distribution area. *G. bicolor* grows in an area around the town of Cruz del Eje between Sierra de Cuniputo to the east and the uplands east of Villa de Soto to the west, to the south it grows south of Cantera Quilpo and at other sites along the banks of Embalse Cruz del Eje water reservoir. *G. bicolor* is ecologically associated with communities of the Chaco Árido formation with *Acacia furcatispina*. In contrast, *G. simplex* grows in communities of open stands with *Acacia caven* conditioned by grazing, often on small rocks accompanied by species of stony substrates at higher altitudes such as *Heterothalamus allienus* (Asteraceae).
The distribution area of *G. simplex* is described above and is larger than that of *G. bicolor*. The distribution areas of the taxa are distinctly separated: the closest locations are at a distance of nearly 80 km. Based on these facts, especially its qualitative character set and distinct distribution area and possibly also its different ecological demands, *G. bicolor* is clearly different from *G. simplex* and deserves the position of a subspecies of *G. mostii* (*G. mostii* subsp. *bicolor* Schütz ex Řepka ined.).

Acknowledgements

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References


