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Djinga cheekii sp. nov. (Podostemaceae) from Cameroon

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A new species is added to the monotypic African genus *Djinga. Djinga cheekii* Ghogue, Huber & Rutish. (Podostemaceae) is described as a new species from Cameroon (Littoral Province) and its morphological affinities and conservation status are assessed. The main distinguishing characters are: stamens 2 (not 1 as in *D. felicis*), flower buds inside spathella strongly inclined (not only slightly inclined as in *D. felicis*), and stems lacking or only up to 6 mm long (not up to >6 cm as in *D. felicis*). A molecular analysis revealed that *D. cheekii* is sister to *D. felicis*, and both together are sister to *Ledermanniella linearifolia* and *L. pusilla* which show completely inverted flower buds inside the spathella, as typical for the large and still artificial genus *Ledermanniella.*

The Podostemaceae (Malpighiales, eudicots) are enigmatic plants which are attached to submerged river rocks, mainly in tropical regions of the Old and New World. They flower and set seed for only a short period after the rainy season, then they die off (Rutishauser et al. 2004, Ruhfel et al. 2011). *Djinga* (with *D. felicis* C. Cusset) was described as a monotypic genus and Cameroonian endemic taxon by Cusset (1987). *Djinga felicis* was first thought to be restricted to the Adamawa ridge of northwestern Cameroon, known from the type locality at the northern slope of Mount Djinga only (western Adamawa, near Tignère). Ghogue, however, found *D. felicis* again at five other places along the Adamawa ridge: 250 km southwest of Mount Djinga in a small area of about 50 km in diameter in the Bamenda highlands: Fundong, Anyanjua, Sabga, Bambili and Fembvang junction on the road Bambui – Belo [GHO2090]. The peculiar morphology of *D. felicis* was analyzed by Ghogue et al. (2009). As part of two collection trips of the first author (Ghogue) during 2004 and 2011 a new taxon was discovered in the Littoral Province, resembling *D. felicis* but showing 2 stamens per flower (not just 1 as in *D. felicis*) and having strongly inclined flower buds inside the spathella (not slightly oblique ones as in *D. felicis*). Molecular analysis (Koi et al. 2012) revealed that the new taxon is sister to *D. felicis*.

Djinga cheekii Ghogue, Huber & Rutish. (Podostemaceae) sp. nov. (Fig. 1–16)

Type: Cameroon. Littoral Province. Mantem River, near Manjo, on the Douala–Nkongsamba highway, 490 m a.s.l., 4°49′00″N, 9°46′00″E, fl., fr. 12 Jan 2011, J. P. Ghogue GHO2125 (holotype: K, isotypes: YA, Z/ZT + spirit).

Etymology

The epithet of the new species honours the British botanist Martin Cheek (Kew Herbarium) for his effort to improve the knowledge on the Cameroonian plants and their threatened status (Onana and Cheek 2012). Cheek is the one who already in 2002 initiated the fruitful collaboration of Ghogue (first author) with botanists from the Univ. of Zurich (Herbaria Z/ZT).

Description

Rheophytic annual herb, submerged in flowing water during wet season. Rooting structures ('roots') as dorsiventrally flattened crusts, attached to substrate (rock) with adhesive hairs on lower surface; crusts 0.3 mm thick and up to 7 mm wide, with exogenously born lobes. Crusts covered with short prostrate shoots, consisting of subulate leaves (Fig. 1–2). Prostrate shoots arise endogenously from the margin and from the upper surface of the crustose root. Stems only up to 6 mm long, unbranched or rosette-like as long as they are not flowering (Fig. 1–3). Leaves up to 4 mm long (rarely up to 7 mm), subulate and flattened laterally (ensiform), arranged in one plane, i.e. in two rows (distichous phyllotaxis); leaf tips occasionally curved downwards towards substrate (Fig. 7, 11); leaves of vegetative shoots usually with a single broad sheath each (Fig. 13–14). Stipules lacking, except for two minute lateral teeth found rarely in uppermost leaves (bracts) next to flowers. Reproductive shoots provided with spathellas, inserted between the subulate leaves (Fig. 4, 15). Each spathella has an ovoid to elliptic sac containing a floral bud. Reproductive shoots start with a terminal flower at the end of the main axis (up to 5 mm long) after the production of up to 20 leaves (Fig. 7–8, 11–12). Additional flowers may

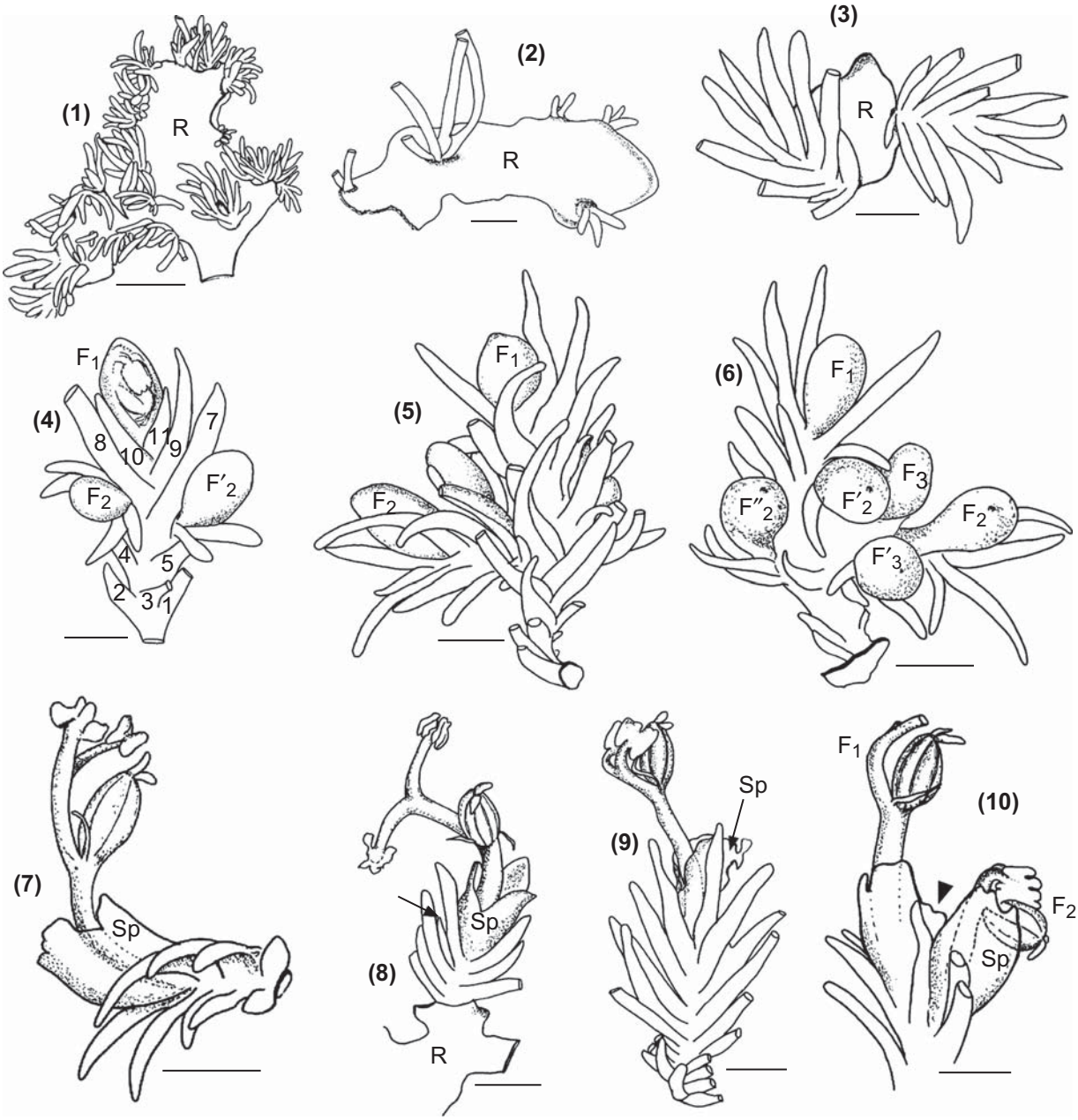

Figure 1–10. *Djinga cheekii* sp. nov. (Podostemaceae) from Cameroon. (1) Mature portion of crustose root (R), covered by short root-born shoots, forming rosettes with subulate leaves. CMR035, scale bar = 3 mm; (2) Young lobe of crustose root (R), with leaf rosettes (short shoots) arising endogenously from root flank and from upper root surface. CMR035, scale bar = 1 mm; (3) Two vegetative shoots arising from crustose root (R). Leaves are subulate and flattened laterally (ensiform) showing distichous phyllotaxis. GHO2125, scale bar = 1 mm; (4) Reproductive shoot with three young flowers, covered by sac-like spathella each. Terminal flower (F_1) at the end of the main axis with its 11 leaves (labelled as 1–11), two lateral flowers (F_2/F'_2) arising from gaps ('axils') below leaf 7 and 8, respectively. Note F_1 with strongly inclined ovary position inside spathella. GHO2125, scale bar = 1 mm; (5)–(6) Reproductive shoot with six young flowers in spathellas, seen from below (substrate) and above, respectively. Flowers labelled according to branching order, with F_1 as terminal flower of primary axis, F_2/F'_2/F''_2 as secondary flowers of lateral shoots and F_3/F'_3 as third-order flowers accompanying a lateral shoot. GHO2125, scale bar = 1 mm; (7) One-flowered prostrate shoot; anthetic flower curved upwards with stalk arising from inside spathella (Sp); subulate leaves curved downwards. Note presence of two anthers (dehisced) on common stalk (andropod); slightly ribbed ovary topped by two linear stigmas. Note presence of two linear tepals at insertion point of andropod and ovary. GHO2125, scale bar = 1 mm; (8) Another one-flowered shoot arising from crustose root (R). Anthetic flower seen from above, with two anthers (dehisced) on common stalk (andropod), with two linear tepals and ovary showing three inconspicuous ribs per valve. Seven leaves in distichous order below spathella (Sp). Arrow points to young leaf of lateral shoot arising between two fully grown leaves. GHO2125, scale bar = 1 mm; (9) Another one-flowered shoot, consisting of ca 20 leaves and terminal stalked flower inserted in withering spathella (Sp). Flower prior to anthesis, with anthers not dehisced. GHO2125, scale bar = 1 mm; (10) Tip of two-flowered shoot, with a terminal flower (F_1) shortly after anthesis (anthers dropped) and a lateral flower (F_2) just leaving ruptured spathella (Sp), with ovary in oblique position and anthers above it. Arrowhead points to the double-sheathed leaf between the two flowers. GHO2125, scale bar = 1 mm.

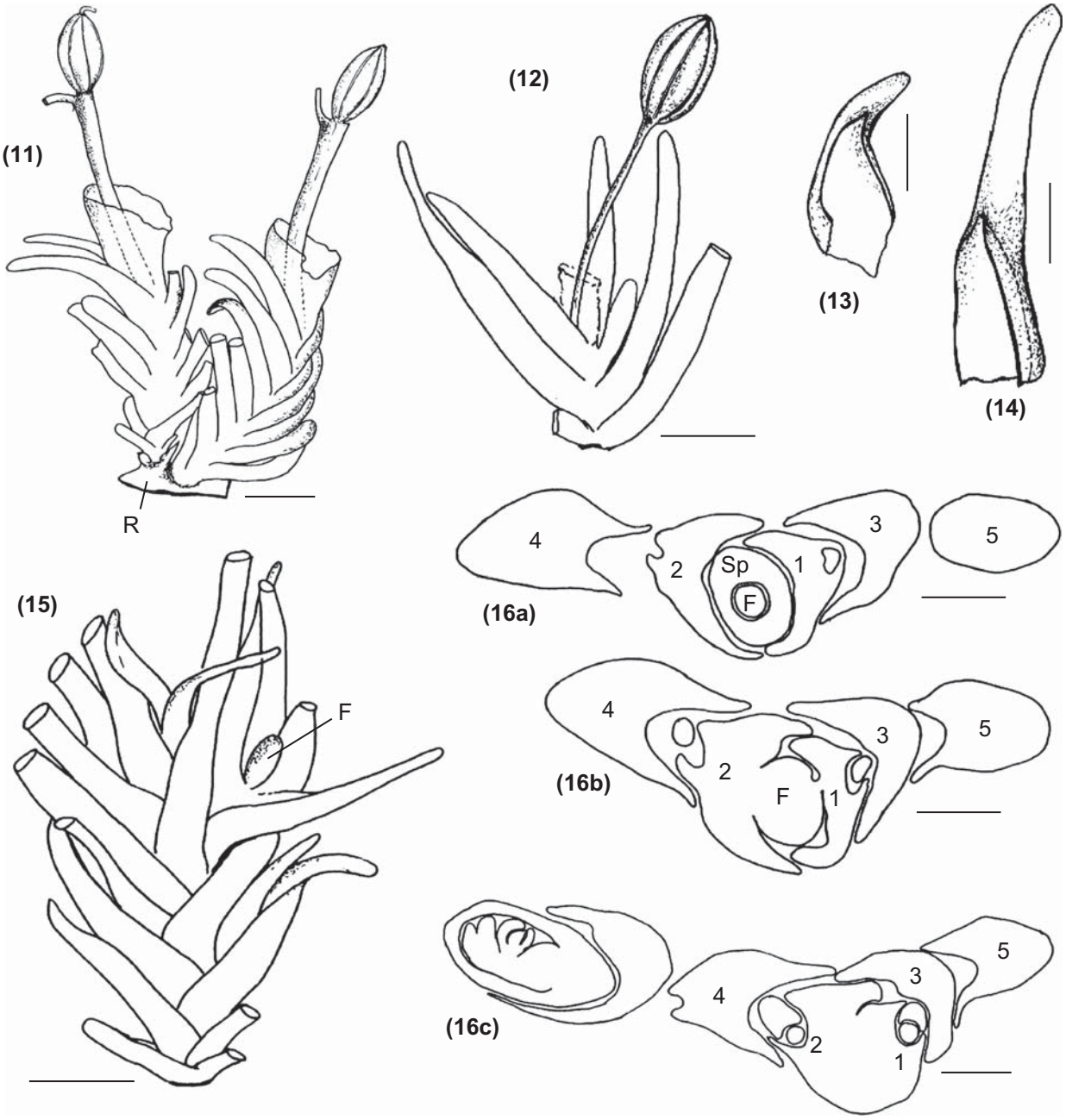

Figure 11–16. *Djinga cheekii* sp. nov. (Podostemaceae) from Cameroon. (11) Two one-flowered shoots arising from same crustose root (R). Leaves arranged in two rows (distichous phyllotaxis), with leaf tips curved towards one side. Flowers after anthesis, with appendages dropped except for young capsule and remnants of ruptured spathellas. Dark lines in drawn capsules indicate furrows. GHO2125, scale bar = 1 mm; (12) One-flowered shoot with mature ribbed capsule, carried on thin stalk (pedicel) after loss of cortical parenchyma. Dark lines in drawn capsule indicate furrows. GHO2125, scale bar = 1 mm; (13)–(14) Two leaves with a single broad sheath each. One leaf with short blade, the other one with elongate, laterally flattened blade. GHO2125, scale bar = 0.5 mm; (15) Branched reproductive shoot prior to anthesis, with most leaves arranged in one plane and only one flower bud (F) observable from outside. GHO2125, scale bar = 1 mm; (16a)–(16c) Three consecutive transversal microtome sections of young reproductive shoot; leaves arranged nearly in one plane. Central flower bud (F) surrounded by spathella (Sp). Leaves 1, 2 and 4 adjacent to flower are double-sheathed, i.e. provided with a cleft or furrow ('second sheath') on the outer ('abaxial') side. New leaves arise from these clefts. Note in (16c) longitudinal section through flower bud inside spathella, with ovary in inclined position. GHO2125, scale bars = 250 μm.

arise from gaps ('axils') between lateral leaves. There are a few third-order flowers in luxurious shoots (Fig. 4–6). Transversal microtome sections (Fig. 16a–c) show that leaves along the main axis are arranged in one plane or nearly so. Some (not all) leaves adjacent to flowers are double-sheathed, i.e. provided with an additional cleft ('sheath') on the dorsal side. Lateral flowers are preceded by 1–3 leaves which are oriented obliquely to the plane of the first-order leaves (Fig. 4–5). Flowers solitary or up to six per shoot. Flower buds are strongly inclined but not

completely inverted in spathella (Fig. 4, 10, 16c). The flower starts to turn upwards into erect position after the spathella is ruptured (Fig. 7–10). Anthetic flowers erect (or nearly so) with 1 mm long stalk (pedicel) arising from inside the spathella. Tepals 2, linear, much shorter than the filaments. Stamen 2; on common stalk (andropod) as long as free filaments, ca 1.2 mm long. Pollen in dyads. Ovary ca 1.2 mm long, ellipsoid, smooth or with three inconspicuous ribs per valve topped by two linear stigmas (Fig. 7–10). Mature capsule carried on a thin stalk (pedicel, up to 3–4 mm long) with cortical parenchyma dropped (Fig. 11–12). Capsules ellipsoid, ca 1.2 mm long, unilocular, opening by two equal or slightly unequal valves; each valve with 3 ribs; ribs somewhat flattened, wider than the furrows; stigmas and other floral appendages usually dropped. Pedicels elongating in fruit, up to 4 mm long (Fig. 11–12).

Distribution and habitat

Cameroon. Littoral Province, near border to Southwest Province. Only known from two localities near Manjo in Mbo River and Mantem River (see 'type/paratypes'). There is a gap of about 200 km distance between the localities of *D. felicis* and the sites where *D. cheekii* grows. Therefore, the two species appear to be clearly disjunct. The area is generally a lowland area, 490 m a.s.l., slope 8–10%. *D. cheekii* grows in more or less rapid water of a clean stream, water temperature 18–20°C, exposed to full sun. Plants fixed on submerged surface of volcanic rocks, or on pebbles of volcanic rocks, and permanently chafed by the stream although turbulence of the stream is lower, as compared to most other Cameroonian Podostemaceae, which grow in more turbulent water.

Conservation status

Djinga cheekii is known from only two sites in Cameroon. Its total area of occupancy (AOO) for these two locations is less than 60 m^2. Both the Mbo river and the Mantem river belong to the Wouri basin. There are food and palm oil farms, bordering the habitats along the rivers next to the sites of the new species. Both sites are suffering from serious pollution, especially from palm oil extraction upstream. *Djinga cheekii* as modular organism fixed to river rocks is here assessed as 'Critically Endangered' (CR) under criterion B2a,b (iii), using the IUCN red list categories and criteria (IUCN 2001, 2010).

Molecular data

Molecular data indicate that most podostemoid taxa of continental Africa belong to one clade (Kita and Kato 2001, Moline et al. 2007, Thiv et al. 2009, Ruhfel et al. 2011, Koi et al. 2012), including *Dicraeanthus*, *Djinga*, *Inversodicraea* (syn. *Ledermanniella* subg. *Phyllosoma* sensu Cusset 1983), *Ledermanniella* s.s. (syn. *Ledermanniella* subg. *Ledermanniella* sensu Cusset 1984), *Leiothylax*, *Letestuella*, *Macropodiella*, *Monandriella*, *Saxicolella*, *Stonesia*, and *Winklerella*. Ruhfel et al. (2011) analyzed three plastid and a mitochondrial marker whereas Koi et al. (2012) added *matK* analyses in many more taxa. They increased support within the clade of African taxa as studied by Moline et al. (2007) and Thiv et al. (2009). Ruhfel et al. (2011) and Koi et al. (2012) found strong support that the genus *Ledermanniella* s.s. as redefined by Thiv et al. (2009) (equalling the former *Ledermanniella* subgenus *Ledermanniella* sensu C. Cusset minus *Monandriella linearifolia* Engler) is not monophyletic. Ruhfel et al. (2011) confirmed an African clade containing taxa whose pollen is shed primarily in dyads. This *Ledermanniella* dyad clade is represented in Ruhfel et al. (2011) by *Dicraeanthus zehnderi* H. Hess, *Djinga felicis*, *Ledermanniella bowlingii* (J. B. Hall) C. Cusset, *Ledermanniella letouzeyi* C. Cusset, *Ledermanniella linearifolia* Engler and *Ledermanniella pusilla* (Warm.) C. Cusset. Koi et al. (2012) confirmed this large *Ledermanniella* dyad clade of Podostemoideae from continental Africa, including *Dicraeanthus*, *Djinga*, and *Ledermanniella* pro parte, whereas *Leiothylax*, *Letestuella*, *Macropodiella*, *Stonesia*, *Winklerella* and another set of *Ledermanniella* spp. formed a second subclade provided with single pollen (*Ledermanniella* monad clade). Within the *Ledermanniella* dyad clade, Koi et al. (2012) found *D. cheekii* (CMR35) as sister to *D. felicis*, and both together were sister to *Ledermanniella linearifolia* and *L. pusilla* (see cladogram in Fig. 17, taken from Koi et al. 2012). Therefore, these four species together may be accepted as members of the so-called *Ledermanniella linearifolia* subclade.

Similar species

Table 1 contains morphological characters of the *Ledermanniella linearifolia* subclade that allows distinguishing between *Djinga cheekii* sp. nov. and its related species *D. felicis*, *L. linearifolia* and *L. pusilla.* The main characters shared by the four species are: rooting structures ('roots') as dorsiventral crusts with exogenous lobes; leaves with narrow bases, arranged in one plane; pollen shed in dyads; capsules slightly anisolobous, 3 ribs per valve, two linear stigma lobes. The majority of African Podostemoideae (including *Djinga* and *Ledermanniella*) have uni-locular ovaries whereas nearly all American and Australasian podostemoids have bi-locular ovaries (Rutishauser et al. 2004, Ghogue et al. 2009).

Djinga cheekii sp. nov. looks similar to *D. felicis*, as described in detail by Ghogue et al. (2009). However, it has always two stamens (n = 50 flowers) whereas *D. felicis* has only one stamen per flower. Moreover, *D. cheekii* has ovaries which are strongly inclined or nearly inverted inside the spathella (i.e. clearly $> 90°$) whereas *D. felicis* has ovaries slightly inclined (i.e. usually $< 90°$).

Djinga cheekii as well as *L. linearifolia* and *L. pusilla* have firm dyads throughout, whereas in *D. felicis* the pollen are arranged in dyads which start to decay into monads during anthesis. Ghogue et al. (2009) found ca 80% dyads and ca 20% monads in nearly mature anthers of *D. felicis*. Unlike typical *D. felicis* (having elongated stems), there are only rosette-like shootlets up to 6 mm in *D. cheekii*, arising endogenously from crustose roots.

There is no doubt that *D. cheekii* is a close relative of *D. felicis*, although the strongly inclined position of the flower buds (ovaries) is intermediate between the slightly inclined position in *D. felicis* and the completely inverted

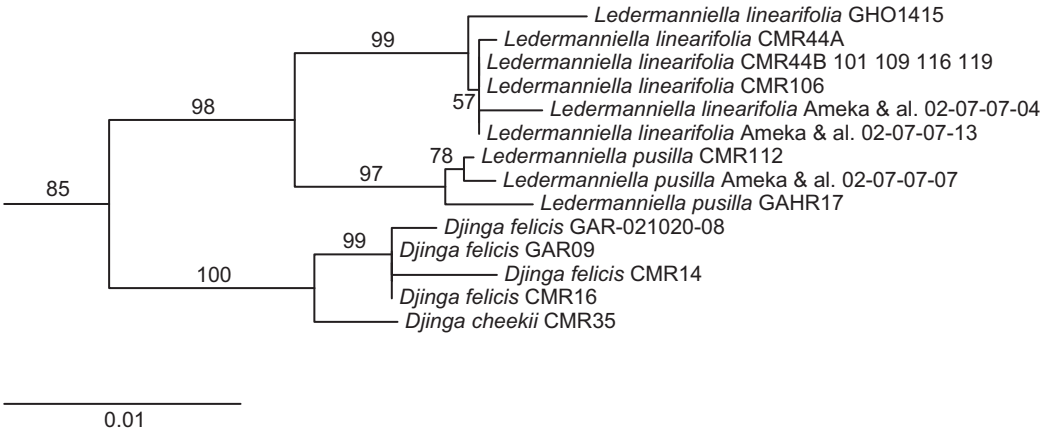

Figure 17. *Ledermanniella linearifolia* subclade with *Djinga* spp. as part of the African *Ledermanniella*-Dyad clade. Phylogenetic tree taken from Koi et al. (2012), deduced from ML analysis of *matK* sequences. Scale indicates substitutions/site. *Djinga cheekii* sp. nov. is sister to *D. felicis*, and both together (forming the genus *Djinga*) are sister to *Ledermanniella linearifolia* and *L. pusilla*.

position in *Ledermanniella* s.s. Despite the fact that the new species has strongly inclined floral buds (inside spathella) we decided to add it (as *D. cheekii*) to the genus *Djinga* instead of putting it to the rather large (and paraphyletic) genus *Ledermanniella* s.s. (i.e. *Ledermanniella* subg. *Ledermanniella* according to Cusset) with its completely inverted floral buds inside spathella. *Ledermanniella* s.s. as a rather large and artificial genus (with ca 25 species) still awaits new classification based on molecular data (cf. Thiv et al. 2009, Koi et al. 2012). In order to avoid paraphyly and to identify the new genera as monophyletic groups, the genus *Ledermanniella* Engler will have to be redefined as a much smaller genus with *Ledermanniella linearifolia* Engler as its type species (Engler 1909).

Table 1. Comparison of morphological characters distinguishing the members of the *Ledermanniella linearifolia* subclade (defined by molecular evidence as shown in Fig. 17, data from Koi et al. 2012).

	Djinga felicis	*Djinga cheekii*	*Ledermanniella linearifolia*	*Ledermanniella pusilla*
Roots	Crusts	Crusts	Crusts	Crusts
Root-born stems (arising endogenously)	Usually branched, up to 60 (rarely 160) mm	Branched or not, up to 6 mm (or stems lacking)	Branched or not, up to 5 (rarely 25) mm	Usually branched, up to 40 mm
Leaf arrangement along stems (or in rosettes if stems are very short or even lacking)	Distichous but leaves of lateral shoots arranged in other planes	Distichous but leaves of lateral shoots arranged in other planes	Distichous with leaves of lateral shoots ± in same plane as mother shoot	Distichous with leaves of lateral shoots in same plane as mother shoot
Leaves [with one sheath each or double-sheathed in the species studied]	Linear and entire (or rarely forked), up to 15 mm	Linear and entire (never forked), up to 2 (very rarely 7) mm	Linear and entire (never forked), up to 20 (–40) mm	Forked once or twice with linear segments, up to 16 (–60) mm
Stipules (if present: two and attached to leaf base)	Lacking or inconspicuous except for uppermost leaves next to flower	Lacking or inconspicuous (rarely as fine teeth on uppermost leaves)	Usually present, also in non-flowering shoots	Usually present, also in non-flowering shoots
Spathella stalk ('peduncle')	Absent, i.e. spathella in bud sessile	Absent, i.e. spathella in bud sessile	Usually present, up to 12 mm long, i.e. spathella long stipitate	Present, up to 13 mm long, i.e. spathella long stipitate
Ovary position in spathella (prior to anthesis)	Slightly inclined to nearly upright, turning upright during anthesis	Strongly inclined, turning upright during anthesis	Completely inverted, turning upright during anthesis	Completely inverted, turning upright during anthesis
Stamen number (with andropod if 2 or 3 present)	1	2	2 (rarely 3)	2 (rarely 3)
Pollen	Mainly dyads (i.e. monads up to 20%)	Dyads	Dyads	Dyads
Tepals	Subulate and shorter than 1/2 ovary length	Usually linear and > 1/2 ovary length	Subulate and shorter than 1/2 ovary length	Subulate and shorter than 1/2 ovary length
Ovary and capsule shape (Length /Diameter ratio);	(Sub-)spherical i.e. L/D = 1.0–1.5	Ovoid–ellipsoid, i.e. L/D = 1.5–2.0	Fusiform (or narrow ellipsoid), i.e. L/D = 2 or > 2	Fusiform (or narrow ellipsoid), i.e. L/D = 2 or > 2
Gynophore (i.e. ovary and capsule with stalk above insertion point of androecium)	Absent, i.e. ovary not stalked, except for presence of pedicel (floral stalk)	Absent, i.e. ovary not stalked, except for presence of pedicel (floral stalk)	Usually present in anthesis, elongating to 0.5 mm in fruit, in addition to pedicel	Usually present in anthesis, elongating to 1 mm in fruit, in addition to pedicel

Therefore, the genus *Ledermanniella* s.s. may turn out to include only *Ledermanniella linearifolia* and *L. pusilla* (besides few other related species not yet included in molecular analyses), whereas *Djinga* (with *D. cheekii* and *D. felicis*) will be its sister genus.

Additional specimens examined (paratypes)

Cameroon. Littoral Province. Mantem River, near Manjo, on the Douala–Nkongsamba highway, 490 m a.s.l., 4°49′00″N, 9°46′00″E, fl., fr. 12 Jan 2011, J. P. Ghogue GHO2126 and 2128 (K, YA, Z/ZT + spirit); Mbo river, Manjo (Manengole village), 4°52′37″N, 9°51′17″E, fl., fr. 12 Dec 2004, R. Imaichi, Y. Kita and J.-P. Ghogue CMR35 (TNS, AB698230; Z/ZT) (Fig. 1–2).

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