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The rediscovery of *Schizostephanus gossweileri* and its phylogenetic position

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Abstract

Two new records of *Schizostephanus gossweileri* (S. Moore) Liede from north-western Namibia and south-western Angola are documented and an amplified description is provided for the species. Analysis of ITS and *trnL*–F data provides additional evidence that this species belongs to *Schizostephanus* Hochst. ex K. Schum., where its relationship to *S. alatus* is strongly supported. The two species of *Schizostephanus* form a lineage that is either unsupported or weakly supported as sister to *Cynanchum* L. s.l.

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1. Introduction

The genus *Cynanchum* L. s.l. consists of around 250 species and has a cosmopolitan distribution in temperate to tropical regions. In Africa some 31 species occur (Liede, 1996). Molecular investigations (Liede and Kunze, 2002; Liede and Täuber, 2002; Rapini et al., 2003) have been carried out using mainly chloroplast *trnL*–F sequences and have neither resolved satisfactorily the relationships within *Cynanchum* s.l. nor led to a clear circumscription of *Cynanchum*. According to Liede and Täuber (2002; Fig. 2, in which both ITS and *trnL*–F sequences were used), *Cynanchum* s.l. consists of a statistically well-supported clade made up almost entirely of Madagascan endemics in which many succulents are included and an essentially unsupported sister-clade containing the type species, *C. acutum* L. Each of these two major clades is a large polytomy in which some groups can be identified, but the relationships between these groups are unknown. The treatment of these various groups has been very inconsistent: thus, although *Glossonema*, *Odontanthera* and *Pentarrhinum* are all nested within the clade containing *C. acutum* (Liede and Täuber, 2002), they have been maintained

as distinct genera (Liede, 2003; Goyder, 2006b; Liede-Schumann, 2006), while *Sarcostemma*, which is nested within the Madagascan clade has sometimes been treated as a separate genus (Liede, 2003) and sometimes as part of *Cynanchum* (Liede-Schumann, 2006). As pointed out by Rapini et al. (2003), the information obtained from analyses so far is inconclusive and so the sinking of *Sarcostemma* into *Cynanchum* is probably premature. *Schizostephanus* was separated from *Cynanchum* and consists of the two species *S. alatus* Hochst. ex Benth. & Hook. f. and *S. gossweileri* (S. Moore) Liede (Liede, 1994, 2003; Liede-Schumann, 2006). The sister-relationship of *S. alatus* to the rest of *Cynanchum* was well-supported in Liede and Täuber (2002), though it was not recovered either in Liede and Kunze (2002) or in Rapini et al. (2003).

Cynanchum gossweileri S. Moore was described from a single specimen collected by John Gossweiler a little before 1911 in Cuanza Norte Province of north-central Angola. Liede (1994) transferred this species to *Schizostephanus* saying, in justification, “The insufficiently known Angolan species *Cynanchum gossweileri* S. Moore shares the long hairs on the adaxial side of the corolla lobes, the stipitate gynostegium and the attachment of the pollinia along a dorsal rim with *S. alatus*. Furthermore, the shape of the guide rails is almost identical in the two species. Despite the fact that latex colour, fruit and seeds are unknown, this species is

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transferred to *Schizostephanus*. *S. gossweileri* is of particular interest because it is the only known case in the Asclepiadaceae where the interstaminal parts of the corona are provided with an adaxial appendage (ligule).”

Until recently, Gossweiler’s collection was the only known material of *S. gossweileri*. In June 2005 a thick-stemmed, at the time leafless, *Cynanchum*-like plant with clear sap was gathered by E. Van Jaarsveld & P. Ems in the Kaokoveld of north-western Namibia and in January 2007 a similar plant was found on an outlier of the Chela Mountains, west of Oncocua in south-western Angola. After careful examination of the type of *S. gossweileri*, we have come to the conclusion that these two specimens belong to *S. gossweileri*.

Here we provide an amplified description of *Schizostephanus gossweileri*. Analysis of ITS and *trnL*–*F* sequences show that it is a close relative of *Schizostephanus alatus*, so that its placement in *Schizostephanus* is probably justified. We also identify certain morphological characters that separate these two species from the rest of *Cynanchum*.

2. Materials and methods

ITS and *trnL*–*F* sequences of 21 representatives of the Cynanchinae (including one species of each of *Glossonema*, *Pentarrhinum*, *Sarcostemma*, as well as one accession of *Schizostephanus alatus* (indicated in Fig. 1 by “FKN”) and 17 species from *Cynanchum*) and three outgroups *Gymnema sylvestre* (Marsdeniaceae, no ITS sequence available), *Funastrium utahense* (Asclepiadeae, MOG clade of Rapini et al. (2003), no ITS sequence available) and *Gomphocarpus physocarpus* (Asclepiadeae, Asclepiadinae) were downloaded from Genbank (sequences as in Liede and Täuber, 2002). Among the species of *Cynanchum*, several of the succulent taxa from Madagascar and mainland Africa were included to match the succulence in *S. gossweileri* and one or two species were chosen from each of the clades found in Liede and Kunze (2002).

ITS and *trnL*–*F* sequences for the two collections of *S. gossweileri* and one new collection of *S. alatus* (McMurtry 13552) were obtained by the protocol outlined in Bruyns and Klak (2006), except that primers ITS4 and ITS5 were used to obtain the whole ITS-sequence. Sequences were assembled and edited using GeneDoc version 2.6.002 and Chromas version 1.43 and aligned by eye. Eight informative gaps were located among the *trnL*–*F* data and ten among the ITS data. These gaps were coded according to the simple gap-coding method of Simmons and Ochoterena (2000). The aligned sequences and gaps were combined into a single matrix. Accession numbers for the new sequences are given in Table 1.

Parsimony analyses were carried out with PAUP* version 4.0b4 (Swofford, 2000), storing at least 1000 trees, using a heuristic search with TBR branch swapping and MULPARS on with all transformations of characters treated as equally likely. To assess support for clades the jack-knife option of PAUP* was used with the percentage of deleted characters set to 36.79%. The outgroup was set to *Gymnema sylvestre*.

Bayesian analyses were carried out with MrBayes 3.1 to find the most likely trees. For these analyses 500,000 trees were sampled from the tree-space with 4 chains running simultaneously, with sampling and printing taking place every 100th tree. The “burnin” was calculated as the first quarter of the 5000 trees sampled so that 1250 of them were discarded and the consensus tree calculated from the remaining 3751 trees. Again, the outgroup was set to *Gymnema sylvestre*.

3. Results

The data contained 1792 characters of which 149 were potentially parsimony-informative (including the 18 gaps). An analysis run with PAUP* without the 18 gaps produced 73 trees. Another analysis run with PAUP* including the gaps yielded 12 trees of length 353 (CI=0.550, RI=0.671, RC=0.369). We show one of these 12 trees as a phylogram (Fig. 1). The two species of *Schizostephanus* are strongly supported as sisters (Jack-knife percentage (JK) 96) on a fairly long branch that is sister to a weakly supported clade made up of the species of *Cynanchum* and including *Glossonema*, *Pentarrhinum* and *Sarcostemma*. This arrangement, where *Schizostephanus* is sister to the rest of *Cynanchum* was present in six of the 12 trees obtained. It is therefore not present in the majority rule tree and it is also unsupported in the Jack-knife analysis. The two collections of *S. gossweileri* are moderately supported as sisters (JK 85), with the South African collection of *S. alatus* weakly supported (JK 70) as sister to *S. gossweileri* and the East African collection as sister to all of them. The very short branches subtending the species of *Schizostephanus* show that their ITS and *trnL*–*F* sequences differ in only a few positions.

In the Bayesian analysis the two species of *Schizostephanus* are strongly supported as sisters (posterior probability (pp)=1.00) and their sister-relationship to the rest of *Cynanchum* is supported (pp=0.85), while *Cynanchum* itself falls into two strongly to weakly supported clades as outlined above (with pp=1.00 and 0.91 respectively). The same arrangement among the accessions of *Schizostephanus* as found under parsimony obtains here. The majority rule consensus tree is not exhibited as it is identical to the phylogram shown as Fig. 1.

Table 1
Accession numbers for new sequences generated for this study.

| Taxon | Origin | Voucher | ITS Accession # | <i>trnL</i> – <i>F</i> Accession # |
|-------------------------------------|-------------------------|---------------------------------|--------------------|---------------------------------------|
| <i>Schizostephanus alatus</i> (McM) | Nelspruit, South Africa | McMurtry 13552 (PRE) | GQ118158 | GQ118159 |
| <i>S. gossweileri</i> (VJ) | Kaokoveld, Namibia | Van Jaarsveld & Ems 18876 (BOL) | GQ118162 | GQ118163 |
| <i>S. gossweileri</i> (PVB) | Chela Mountains, Angola | Bruyns 10677 (BOL) | GQ118160 | GQ118161 |

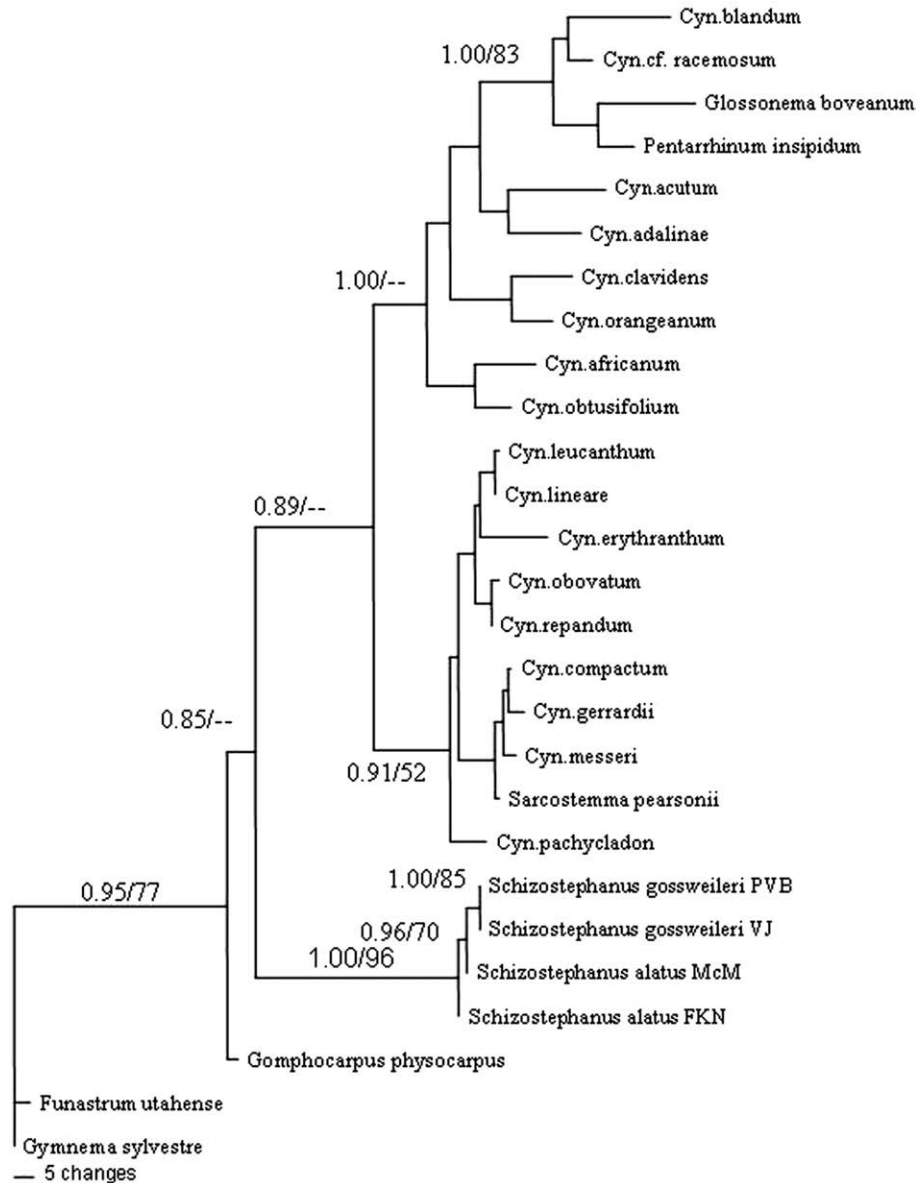


Fig. 1. Phylogram of one of six trees obtained from parsimony analysis of ITS and *trnL*-F data. This tree was also obtained as the consensus tree from Bayesian analyses. Where the support was significant, support values for branches are given near them in the form "Posterior probability (from Bayesian analyses)/Jack-knife percentage".

Schizostephanus gossweileri (S. Moore) Liede in Bot. J. Linn. Soc. 114: 92 (1994). *Cynanchum gossweileri* S. Moore in J. Bot. 50: 363 (1912). Type: Angola, Cuanza Norte, banks of River Luinha, between Luinha and Canhoca railway station, *Gossweiler* (holo., BM!).

Spreading deciduous shrub to climber with clear sap and fibrous roots; *stems*: ascending–spreading and forming a shrub to 1.5 m tall and broad or climbing to 3–4 (10) m, 5–15 mm diam., young stems finely brown-tomentose and bright green, glabrescent and becoming pale grey covered with corky bark. *Leaves*: decussate, ovate-cordate, acute, 30–60 × 25–50 mm, bright green, sparsely pubescent when young, later glabrous, flat, petioles 20–40 × 2 mm, finely pubescent when young, glabrescent. *Inflorescences*: many, alongside each node in upper parts of younger

stems, racemose, each with peduncle 30–80 × 1.5–2 mm, green and finely white-tomentose, lower 10–30 mm without flowers, above with many flowers in groups of 1–2 along peduncle (each group subtended by single small ovate to lanceolate bract 1–1.5 mm long) and opening in gradual succession; pedicel ascending, 4–6 × 1 mm, green, pubescent; sepals ovate-deltate to oblong, 1.5 × 1 mm, obtuse, adpressed to corolla, pubescent, green. *Corolla*: rotate, 7–8 mm diam., divided nearly to base, outside pale green, glabrous, inside yellow becoming maroon in lower third of lobes where with fine scattered hairs up to 0.25 mm long; *tube* ± absent; *lobes* spreading and slightly twisted to right towards tips, lanceolate to oblong-lanceolate, 2.5–3.5 × 1.5 mm at base, obtuse, with eciliate recurved margins. *Corona*: consisting of two series of lobes, 2–3 × 2.5–3.5 mm, glabrous; *outer lobes*

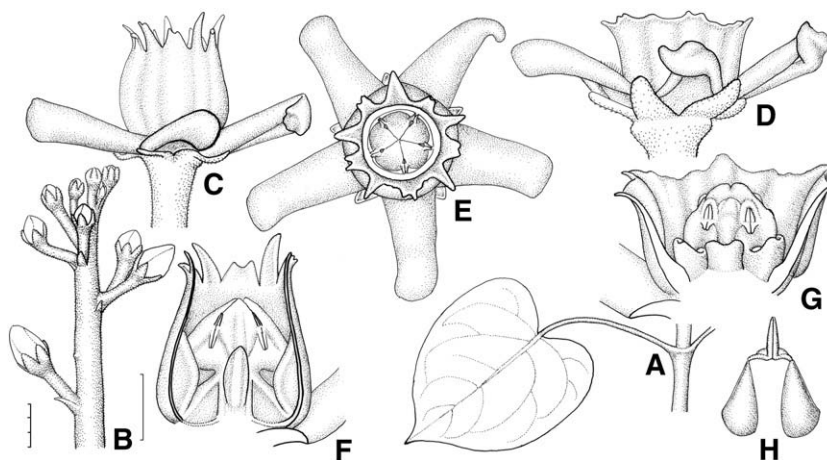


Fig. 2. *Schizostephanus gossweileri* (A, B, C, E, F, H from Bruyns 10677; D, G from Van Jaarsveld & Ems 18876). (A) piece of young stem with leaf; (B) upper part of inflorescence; (C, D) side view of flower; (E) face view of flower; (F, G) side view of centre of dissected flower; (H) pollinarium. Scale bars: (A) 15 mm (at B); (B) 3 mm; (C, D, E) 1 mm (at B); (F, G) 1 mm (at F); (H) 0.25 mm (at B).

fused into urceolate to bowl-shaped tube 2–3 mm long, emarginate to with 10 small lobules around mouth; *inner lobes* up to 1 mm long, arising behind anthers and touching their bases, keeled and obtusely subulate to shortly rectangular and truncate. *Gynostegium* distinctly narrowed below anthers and sometimes with stripe broadening towards base of outer coronal tube, apical sterile appendages of anthers much shorter than to completely covering obtuse and slightly bifid style apex. *Fruit*: a pair of horizontally spreading flattened glabrous green follicles 60–90 × 12–20 mm; *seeds* numerous, 6–7 × 2 mm, with coma of fine hairs 25 mm long at micropylar end. Fig. 2.

Schizostephanus gossweileri is known from two areas, of which one lies in Cuanza Norte (north-central Angola), north of the Cuanza River along the Luinha River. The other is in south-western Angola and in the adjacent high parts of the Kaokoveld of Namibia. Nothing is known about the habitat in which Gossweiler collected this species in Cuanza Norte. Near Oncocua in south-western Angola and the Kaokoveld of Namibia it is associated with steep mountain slopes, growing among rocks at the base of cliffs or among rocks above the cliffs. Near Oncocua a few plants were observed sprawling down the lower parts of some cliffs but most were among large rocks at the base of these cliffs.

With its clear sap and thick stems with deciduous leaves, *S. gossweileri* is unlike any other species of *Cynanchum* in Africa. However, it shares many morphological features with *S. alatus*. These include the succulent stems with clear sap and the prominent, cordate, deciduous leaves. The racemose inflorescence is similar in both, in which flowers develop successively along the peduncle towards its apex. Although similar structures are known in a few other species of *Cynanchum* (as for example in *C. gonoloboides* Schltr.) the inflorescence in most species of *Cynanchum* is umbellate. Specimens of *S. alatus* and both of the new collections of *S. gossweileri* possess hairs on the inside of the corolla lobes near their bases. Moore (1912) gave the corolla lobes of *C. gossweileri* as glabrous but in fact they are also pilose

inside near their bases on the type. Liede (1993) described these as “extremely long and slender, 1–1.5 mm” but the Fig. 17b (Liede, 1993) shows them to be only around 0.5 mm long and they are much shorter even (± 0.25 mm) in the new collections of *S. gossweileri*. Nevertheless, the presence of hairs inside near the base of the lobes is unusual for *Cynanchum* and is more typical of such genera as *Astephanus* (Bruyns, 2003) and *Microloma* (Bruyns and Linder, 1991) where, however, these hairs are stiffly retrorse. In both species of *Schizostephanus* the inner edge of the pollinium (relative to the anther that produced it) is furnished with a faint pellucid margin or possible insertion crest. This is absent from all other species of *Cynanchum* in Africa (Liede, 1996), though it is known also in the two species of *Pergularia* (Goyder, 2006a). Liede (1993, 1994) refers to the “insertion of the pollinium along a dorsal rim” in *S. alatus* but in fact the pollinium is joined apically to the caudicle as in most other Asclepiadeae and it is the pellucid margin that extends down along the inner edge from this join.

Moore (1912) described the corona of *S. gossweileri* as 10-lobed in the upper third, prominently 5-keeled inside and filled at the base with five ovate, entire or bilobed blades alternating with the keels. In the present two collections the corona is a variable structure but consists of two series of lobes. The outer series is fused into an urceolate to bowl-like tube that is variably fluted (or keeled) on the inner surface and divided into 10 lobules or teeth around its apex. Behind each anther there is an inner corona lobe which rises up towards the back of the anther. These inner lobes are also very variable in shape. The anthers are borne on a swollen style-head beneath which the gynostegium becomes briefly slender and then broadens out towards the base of the outer coronal tube. The arrangement of the corona is rather similar to that in *Sarcostemma* (see Endress and Bruyns, 2000; Fig. 11 and discussion) except that here the outer series is the larger of the two and dwarfs the inner series of lobes. Such structures on the inside of the outer coronal cup and opposite the anthers are not unknown in *Cynanchum* either, for they are present in *C. virens* (E. Mey.)

D. Dietr. and in *C. gerrardii* (Harv.) Liede. Thus, the claim (Liede, 1994) that the coronal structure of *S. gossweileri* is unique among the asclepiads is erroneous.

Moore (1912) compared *S. gossweileri* with *C. schistoglossum* Schltr. This relationship does not appear to be close and *C. schistoglossum* is a small-flowered species with very different stems, leaves, inflorescence, corona and pollinia, whose relationships within *Cynanchum* are unknown. The molecular analysis shows that *S. gossweileri* is closely related to *Schizostephanus alatus*. Useful morphological characters defining this relationship are the swollen stems and prominent, cordate, deciduous leaves, the clear sap, the hairs on the inside of the corolla lobes near their bases and the pollinia widening towards the base with a pellucid margin along its inner edge.

3.1. Material examined

ANGOLA—0914 (Cuanza Norte), Luinha River between Luinha and Canhoca (Canhaca) railway station, *Gossweiler* (BM). 1613 (Cunene) steep south-facing slopes west of Oncocua (–CA), 1300 m, *Bruyns 10677* (BOL).

NAMIBIA—1712 (Posto Velho): Kaokoveld, mountaintop west of Otjihipa Mountains and east of Hartmann's Valley (–BC), 1900 m, 13 July 2005, *Van Jaarsveld & Ems 18876* (BOL).

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