

A phylogenetic and morphological study of *Polystachya* sect. *Superpositae* (Orchidaceae) with description of a new species from Cameroon

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Abstract *Polystachya* section *Superpositae* is a group of montane and submontane African epiphytes with thirteen species (including the newly described in this paper). During a scientific expedition to Bamenda Highland, Cameroon a new species, *Polystachya bamendae*, was found and it is described here. The new entity was compared with 398 herbarium specimens representing the section, including the type collections. The molecular analyses of the ITS and *matK* strongly support the results of morphological studies and reveal close relationship between the new species and the species of the section *Superpositae*. Two hypotheses supporting function of the *matK* gene in the examined species are presented. The phylogenetic and morphological relation of *Polystachya bamendae* to *P. lindblomii* and *P. superposita* is discussed. The distribution range of the section is analyzed and a discussion about the distribution of the species is presented. A distribution map of the section and dichotomous key to the species of *Superpositae* are provided. The occurrence of the species of the section in two Biodiversity Hotspots (Guinea Forests of West Africa and Eastern Afromontane) is discussed.

Keywords Africa · ITS · *matK* · Morphology · Taxonomy

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Introduction

The genus *Polystachya* Hook. includes 233 species worldwide, of which nearly 90 % occurs in Africa (Mytnik-Ejsmont 2011). Representatives of the genus are sympodial, epiphytic, lithophytic or occasionally terrestrial herbs with terminal inflorescences growing always out of the top of the pseudobulb, with non-resupinate flowers. The flowers, predominantly small, are characterized by a mentum, which is a fusion of lateral sepals and the column foot, and three-lobed lip with usually oblong callus.

The section *Superpositae* Kraenzl. is one of 13 sections of *Polystachya* (Mytnik-Ejsmont 2011). It was described by Kraenzlin in the first monographic treatment of the genus (1926). The representatives of the section are montane and submontane epiphytes characterized i.e., by unique vegetative characters—fusiform pseudobulbs united in an erect or pendulous sympodium, each succeeding one arising from the middle or apical part of the preceding one, 2–8-leaved, short unbranched or paniculate inflorescence, free part of gynostemium 1–2.5(5) mm high, and prominent column foot 4–5 mm long.

The section is divided into three subsections (Mytnik-Ejsmont 2011). The nominal subsection includes five species, *P. eurygnatha* Summerh., *P. fusiformis* (Thouars) Lindl., *P. simplex* Rendle, *P. stewartiana* Geerinck, and *P. superposita* Rchb.f. They are plants with pseudobulbs 3 mm in diameter at least, narrowly elliptic leaves, paniculate, 20–80-flowered inflorescence, lip shortly and broadly clawed, most often without any callus. Within the subsection *Spatellae* Mytnik there is classified seven species, *P. spatella* Kraenzl., *P. ruwenzoriensis* Rendle, *P. lindblomii* Schltr., *P. pudorina* P.J.Cribb, *P. tridentata* Summerh., *P. aethiopica* P.J.Cribb and newly described *P. bamendae* Szlachetko, Baranow & Mytnik. They can be

defined by possession of slender stems up to 3 mm in diameter, scarcely thickened, narrowly oblong ligulate to linear leaves, unbranched inflorescence 1–16-flowered (most often consisting of 3–10 flowers), and long-clawed lip furnished with a fleshy callus. The subsection *Kermesinae* Mytnik is a monotypic taxon represented by *P. kermesina* Kraenzl., one of the showiest species within the subtribe. The species is atypical for *Superpositae* for its flower color and lip structure, however, due to its superposed, slender, 2–3-leaved pseudobulbs, linear leaves, short 3–5-flowered inflorescence, gynostemium structure and distribution range as well, Mytnik-Ejsmont (2011) decided to keep *P. kermesina* in *Superpositae*.

The section was revised once by Cribb (1979). The author presented a synopsis of the group and proposed three informal subgroups within the section. He included six species to the first group (*P. spatella*, *P. ruwenzoriensis*, *P. lindblomii*, *P. pudorina*, *P. tridentata*, and *P. aethiopica*), they were characterized by slender cylindrical stems scarcely pseudobulbous, narrow, grass-like leaves, medium-sized flowers, unbranched inflorescence, and the long-clawed lip furnished with a fleshy simple or lobed porrect callus. The plants with stouter pseudobulbs, broader, narrowly elliptic leaves, a paniculate inflorescence, and small flowers with a shortly clawed lip with or without a small unlobed callus were included in the second group (*P. simplex*, *P. eurynatha*, *P. fusiformis*, and *P. superposita*). The last monotypic group was represented by Malgasian *P. oreocharis* Kraenzl.

The latter species, however, was excluded from the section by Mytnik-Ejsmont (2011). It was classified within *Superpositae* by many authors (Kraenzlin 1926; Cribb 1979, 1984) though it is a completely odd taxon. This Malgasian endemic is characterized by non-pseudobulbous stems completely covered by the imbricating leaf bases, many distichously arranged leaves, geniculate lip with strongly undulate margins and horn-shaped basal callus.

Apart from *Polystachya superposita* and *P. bamendae*, the novelty described in this paper, being endemic to Cameroon Highlands and *P. fusiformis* being widely distributed throughout tropical and subtropical Africa and Madagascar, vast majority of the species of *Superpositae* are confined to East African Rift System (Eastern Afro-montane Biodiversity Hotspot), which is a center of high concentration of the species of the section. They occur mainly at higher elevations, most often from 850 to 3,200 m above sea level from Ethiopia to Mozambique. Five East African species of the section (*P. eurynatha*, *P. kermesina*, *P. ruwenzoriensis*, *P. stewartiana*, and *P. tridentata*) are confined in their distribution to the Albertine Rift (=Western Rift Valley), the western branch of the East African Rift. The Albertine Rift, the region of a large number of endemic species, has been identified as an ecoregion by the World Wildlife Fund and a Biodiversity

Hotspot by Conservation International (2005). Another representative of the section, *Polystachya pudorina*, is an endemic to part of the eastern branch of the East African Rift, the Usambara Mountains, which are unique ancient mountain region covered with the tropical forest. Due to a lack of glaciations and a relatively consistent climate, the rainforest of Usambaras has gone through a long-term evolution resulting in an impressive amount of endemism (Lovett 1993). *Polystachya simplex* and *P. lindblomii*, occur in both branches of the East African Rift along its whole length, from Ethiopian Highlands to Mozambique. *Polystachya spatella*, though known in both Western and Eastern Rift Valley, is confined in its distribution to the region of Victoria Lake. The species is one of the very few polystachyas growing above 3,000 m.a.s.l. It was even found above the evergreen forest belt, in the bamboo zone in Ruwenzori Mountains. The only species occurring exclusively in Ethiopia is *Polystachya aethiopica*, being found in two ecoregions (WWF 2012): the Ethiopian montane forests (up to 1,800 m elevation) and the Ethiopian montane grasslands and woodlands (above 1,800 m).

Today the section includes thirteen species (including the new entity). The new species described here was found during a scientific expedition to Cameroon, in which Szlachetko and Baranow have been conducted taxonomic research of Orchidaceae. The country is among the top ten countries in Africa for biodiversity due to its montane forests, where a high number of endemic plant species occur (BirdLife International 2010) and to its strong latitudinal gradient of precipitation. Now the section *Superpositae* is represented by three species in Cameroon.

Material examined

Morphological study. The study presented here is based on the examination of the plant material collected in the field and 398 herbarium specimens from fifteen herbaria: B, BM, BR, BRLU, C, HBG, K, L, MA, MO, P, UGDA, W, WAG and YA (acronyms according to Thiers 2008, continuously updated). We observed some living plants in WAG and C. The specimen representing the new species was found in south-east of the Bamenda Highlands, about 2 h walking from the village Big Babanki to Czech Field Station.

DNA material. DNA sequences were downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). Two data matrices, ITS and *rps16-matK* were taken from PopSet database no 298263940 (Russell et al. 2010a) and 270484601 (Russell et al. 2010b), respectively. The *rps16-matK* data matrix was trimmed for part of *matK* gene only and then analyzed. Four species (*Adrorhizon purpurascens*, *Sirhookera lanceolata*, *Bromheadia finlaysoniana*, and *B.*

srilankensis) were selected as outgroup for *matK* and two species (*Adrorhizon purpurascens* and *Sirhookera lanceolata*) were selected as outgroup for ITS. In addition, three species (ITS) were taken from GenBank: *Polystachya spatella* HM018555, *P. campyloglossa* HM041027, *P. lawrenceana* HM018549. One species (ITS), *P. fusiformis* (id 068) was taken from data matrix from Anton Russell. Plant material for new species was collected during field expedition in 2011 to Cameroon. DNA from this specimen was extracted from silica dried leaves (Chase and Hillis 1991) using DNA easy Plant Mini Kit (A&A Biotechnology) following manufacturer protocol. ITS and part of *matK* gene (approximately 1,300 bp) were sequenced. Sequences were deposited in GenBank under accession number KC339535 (*matK*) and KC339536 (ITS).

Methods

DNA amplification and sequencing. Polymerase chain reaction (PCR) amplifications were carried out in a total volume of 25 μ l containing 2.5 μ l 10 \times buffer, 1 μ l 25 mM MgCl₂, 1 μ l 5 mM dNTPs, 0.5 μ l of 10 μ M of each primers and 1 U of Red Perpetual DNA polymerase (Eurx, Poland) and H₂O. The ITS region (ITS1-5.8S-ITS2) was amplified via PCR using the primers 101 AB and 102 AB (Douzery et al. 1999), the *matK* was amplified with the following two primers: 19F (Molvray et al. 2000) and 1,326R (Cuénoud et al. 2002). The PCR cycling conditions for ITS and *matK* were template premelting at 94 °C for 3 min followed by 30 cycles of denaturation at 95 °C for 45 s, primer annealing at 52 °C for 1 min, followed by primer extension at 72 °C for 90 s. Final extension step was of 7 min at 72 °C. Amplified products were cleaned with High Pure PCR Product Purification Kit (Roche Diagnostic GmbH, Mannheim, Germany) following manufacturer protocol. Cycle sequencing was carried out using Big Dye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., ABI, Warrington, Cheshire, UK) with the same primers used for PCR amplification: 2.0 μ l of 5 \times sequencing buffer, 1 μ l of Big Dye terminator with 1.5 μ l of 10 μ M primer (1.5 pmol), 1–2 μ l of amplified product (30–90 ng/ μ l), and H₂O in a total of 10 μ l reaction volume. Cycle sequencing conditions were as follows: 25 cycles each with 15 s denaturation (94 °C), 5 s annealing (50 °C), and 4 min elongation (60 °C). The sequences were generated on an ABI 3720 automated capillary DNA sequencer. Both strands were sequenced to assure accuracy in base calling. Sequence Navigator (ABI) was used to edit the sequences, and the two complementary strands were assembled using AutoAssembler (ABI).

Sequence alignment and phylogenetic analyses. The ITS and *matK* sequences were aligned by eye using Seaview

(Galtier et al. 1996). Maximum parsimony analyses were undertaken on two matrices separately and combined analyses using heuristic searches in PAUP* version beta 10 (Swofford 2000) with tree-bisection-reconnection (TBR) branch swapping and the MULTREES (holding multiple trees) option in effect with 1,000 replicates of random sequence addition, but saving only 10 trees for each replicate to reduce the time spent in swapping large numbers of suboptimal trees. Standard tree parameters were estimated (tree length, consistency index, CI, and retention index, RI). Internal support of clades was evaluated using non-parametric bootstrapping (Felsenstein 1985) with 1,000 replicates and the same settings as above, except for simple sequence addition. All characters were treated as unordered and equally weighted (Fitch 1971).

Morphological study. Standard procedure of preparing the herbarium material to facilitate stereomicroscopic observation was applied. The following vegetative characters of individual plants were analyzed: stem (height, shape, presence of glandular hairs), leaves (number, size, shape), sheaths (number, shape, size), inflorescence (size, density), floral bracts (size, shape, presence of glandular hairs) flowers, taken from the middle part of the inflorescence (presence of glandular hairs, size of pedicel and ovary, height and shape of mentum (spur), size and shape of lateral sepals, dorsal sepal, petals, and lip), as well as gynostemium. Particular parts of the flower were boiled, dissected, measured and drawn under a stereomicroscope. The results were then analyzed and compared with the type material, diagnoses and original illustrations. The database of the drawings and photographs of all studied specimens is available in the first author's archives. For all species type material was available.

Results

Statistics for ITS and *matK* and combined data matrices are separated by “/”. The number of analyzed taxa was 75/129/15, respectively. The aligned matrix comprised 860/1,326/2,158 characters of which 295/263/295 were variable and 195/162/120 were potentially parsimonial informative. The number of the most parsimonious trees were >10,000 for ITS and *matK* and 3 for combined analysis. Tree length was 702/414/360, consistency index (CI) = 0.6/0.7/0.7, and retention index (RI) = 0.8/0.9/0.8. One of the most parsimonious trees is depicted in Figs. 1, 2 and 3. Bootstrap support (BS) \geq 50 % is given for supported clades below branches. Clades that collapse in the strict consensus tree are marked by an arrow. Both analyses indicate close relationships between new species and *Polystachya* section *Superpositae*. The bootstrap support for mentioned clade is low (BS <50/=50/56). Except *P. lindblomii*, all sequences from the *Superpositae* clade in *matK* gene possess 19 bp

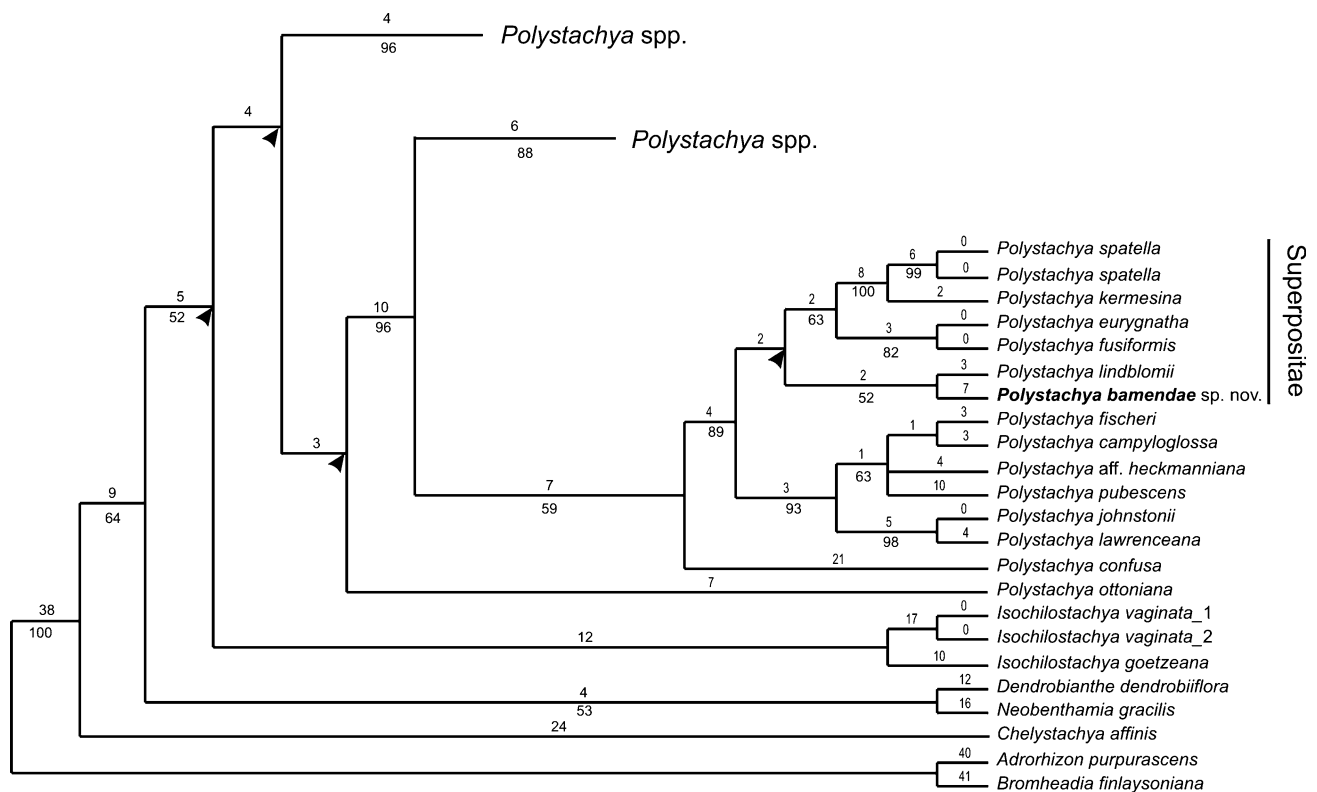


Fig. 1 One of the most parsimonious trees in ITS analysis ($L = 702$, $CI = 0.6$ and $RI = 0.8$) for Polystachyinae. New species is highlighted in **bold**. The numbers above branches are Fitch branch lengths.

Bootstrap percentages >50 are given for supported clades below branches. *Arrows* indicate clades that collapse in the strict consensus tree

indel (duplication event). The indel occurrence causes a change of frameshift and as a natural consequence, an activation of the stop codon. The movement of the frameshift by one nucleotide downstream in the species possessing the indel enables an obtainment in silico the amino-acid sequence, which is homological to the sequences of the rest of the species and it does not have the stop codon within the gene sequence.

Taxonomic treatment

Polystachya Hook. sect. *Superpositae* Kraenzl. subsect. *Spatellae* Mytnik

Polystachya bamendae Szlachetko, Baranow & Mytnik, sp. nov.

Type: Cameroon. North West Province: Bamenda Highlands about 2 h walking from the village Big Babanki to the Czech Field Station, in degraded secondary forest, ca 2–3 m above the ground on *Eucalyptus* trunk, elev. 2,150 m, 11–26 Nov 2011, fl. Sep–Oct 2012, *Szlachetko & Baranow* 9465 (holotype: UGDA; drawing of the holotype: BRLU, K, YA).

Polystachya bamendae is related to *P. lindblomii*, but the former species has the fractiflex rachis, prominent lip lateral lobes and the lip middle lobe 3 times wider than long.

Etymology: In reference to the place where the type specimen was collected.

En epiphytic plant up to 20 cm long. Pseudobulbs 7–11 cm long, 0.3–0.6 cm in diameter, fusiform, each succeeding one arising from the middle or apical part of the proceeding one. Leaves up to 6.5 cm long, 0.7 cm wide, narrowly lanceolate to linear-lanceolate, shortly acuminate. Inflorescence up to 8 cm long, branched, 4–20-flowered; rachis fractiflex, pubescent. Floral bracts 3 mm long, 4 mm wide, caudate. Flowers sparsely pubescent, fleshy, green with white apices of petals, lip yellow-white with yellow callus in the center. Pedicel and ovary 3–4 mm long, sparsely pubescent. Dorsal sepal 4 mm long, 2.8 mm wide, oblong-elliptic, caudate, apex 1.5 mm long. Lateral sepals 4.5–5 mm long, 2 mm wide, saccate, lance-ovate, caudate, apex 1–1.5 mm long. Menthum (spur) deeply bilobed, up to 4 mm long, 2 mm wide, rounded, saccate. Petals 3.5 mm long, 1 mm wide, narrowly elliptic, acute. Lip 4.2–6 mm long, 4–6 mm wide, 3-lobed, glabrous, strongly fleshy in the middle, squeezed in the middle on the outer surface,

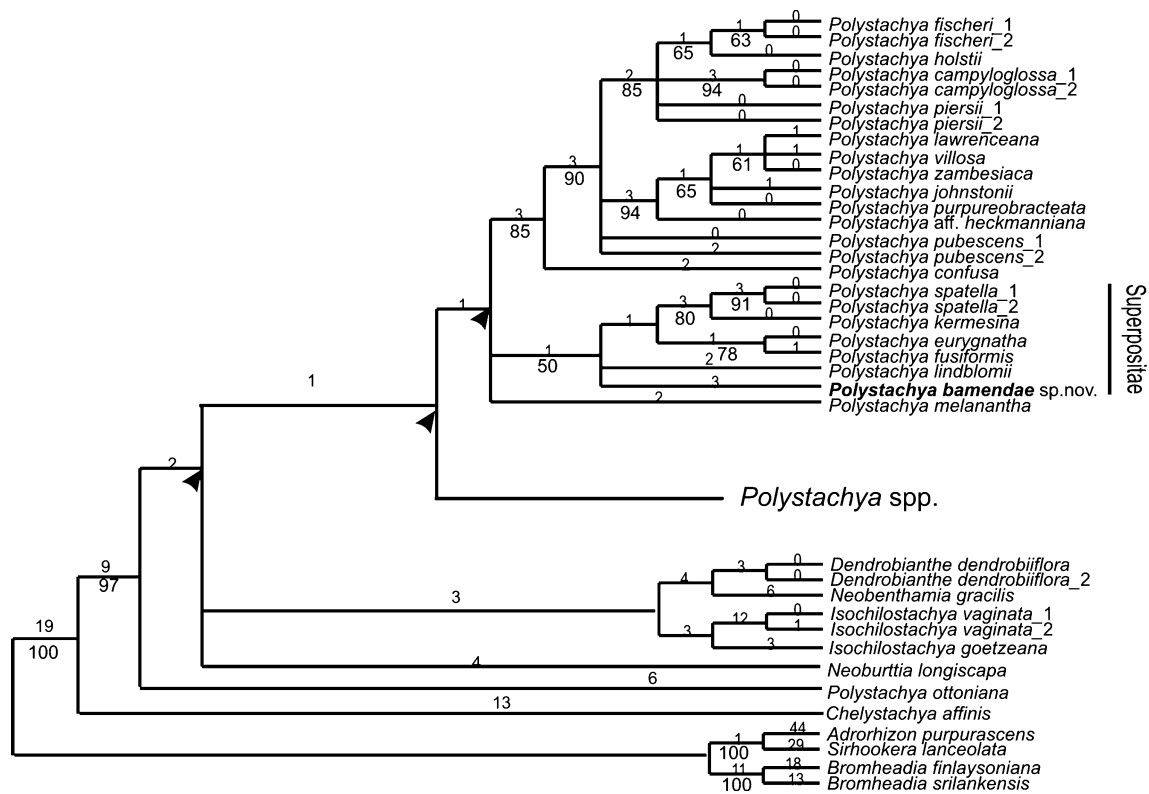
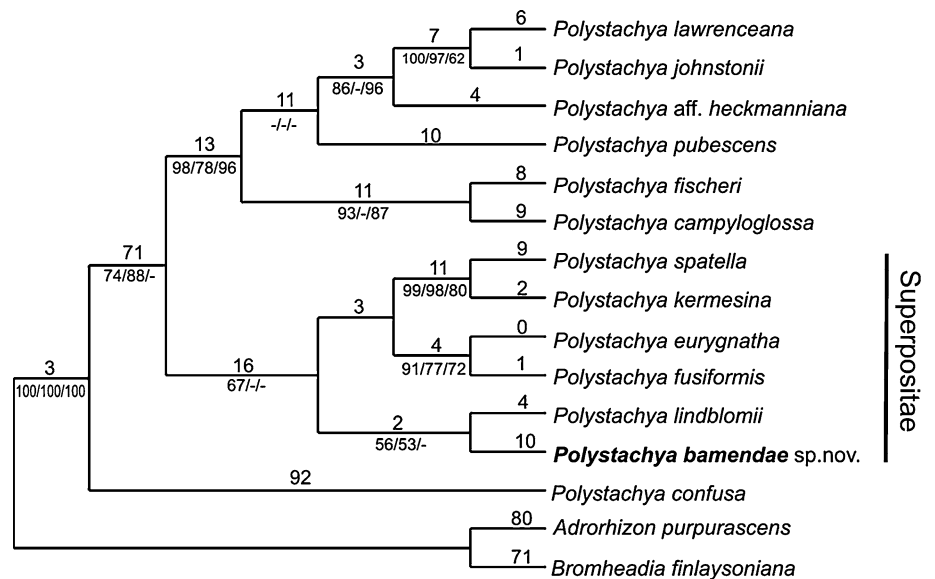


Fig. 2 One of the most parsimonious trees in *matK* analysis ($L = 414$, $CI = 0.7$ and $RI = 0.9$) for Polystachyinae. The new species is highlighted in *bold*. The numbers above branches are Fitch

branch lengths. Bootstrap percentages >50 are given for supported clades below branches. *Arrows* indicate clades that collapse in the strict consensus tree

Fig. 3 One of the most parsimonious trees in combined (*matK* + ITS) analysis ($L = 360$, $CI = 0.8$ and $RI = 0.9$) for Polystachyinae. The new species is highlighted in *bold*. The numbers above branches are Fitch branch lengths. Bootstrap percentages >50 are given for supported clades below branches—combined/ITS/*matK*, respectively. *Arrows* indicate clades that collapse in the strict consensus tree



unguiculate, claw 2.5 mm long, 1.8 mm wide, ribbon-like, strongly fleshy along the midnerve; lateral lobes 2–2.5 mm long, 1.3–20 mm wide, widely obovate, obtuse, widely divergent; the middle lobe 2 mm long, 4.5–6 mm wide, transversely widely oblong, crenulate on margins, 4-lobed, emarginate with an apex 0.5–0.6 mm long on outer

surface; the callus 1–2 mm long, 1 m high, fleshy bilobed ridge along the midnerve in the middle of the lip. Column foot 2–2.5 mm long, column 1.4–2 mm long, stout, anther 1.1 mm long and wide, beak-like, anther partitions prominent, lemon-shaped, 0.5 mm long, 0.3 mm wide; rostellum remnant retuse (Fig. 4).

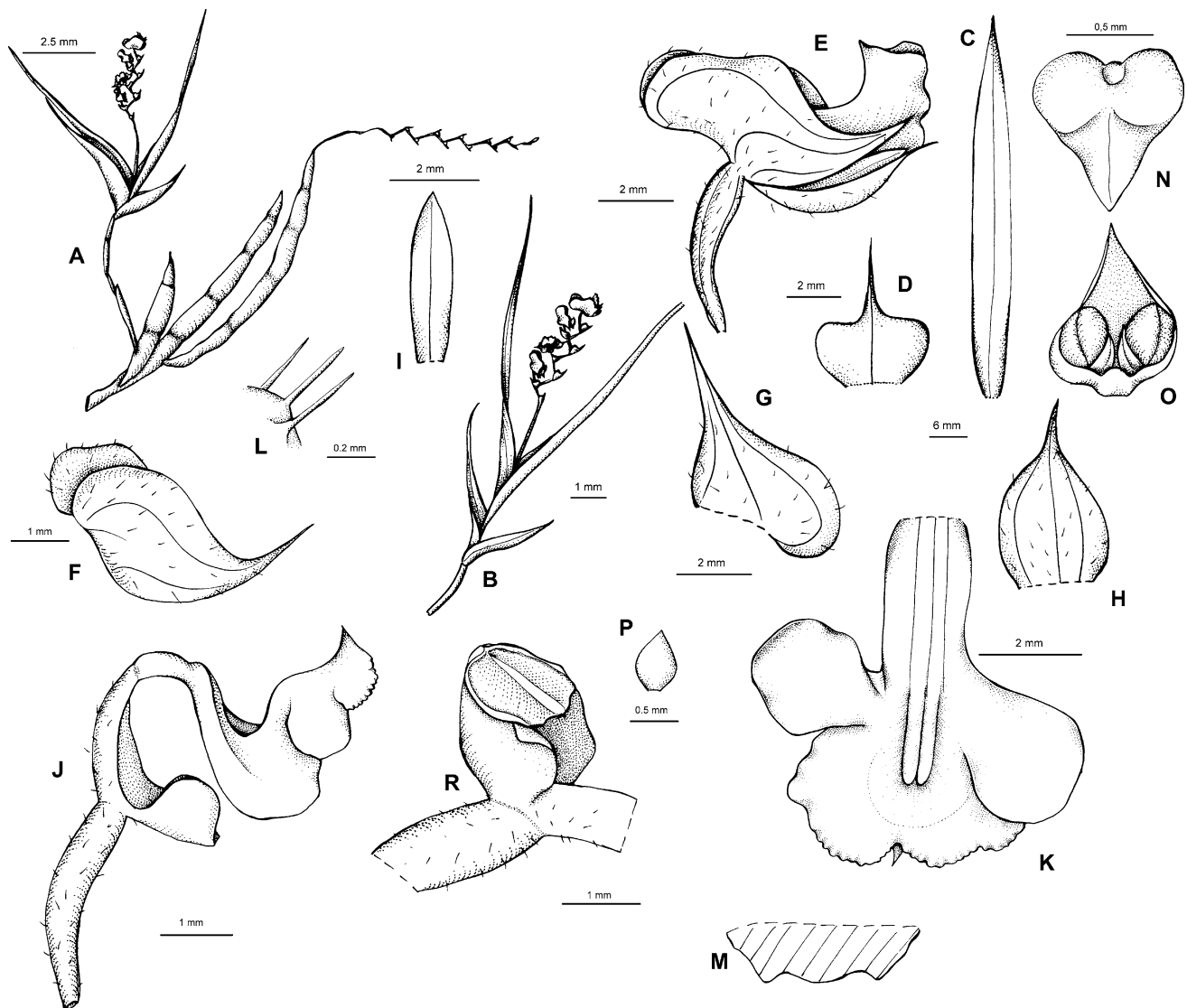


Fig. 4 *Polystachya bamendae* Szlachetko, Baranow & Mytnik **a** habit, **b** inflorescences and leaves, **c** leaf, **d** floral bract, **e** flower, **f** mentum, **g** lateral sepal, **h** dorsal sepal, **i** petal, **j** flower lacking

lateral sepals, **k** lip, **l** trichomes on sepals, **m** rostellum remnant, **n** anther top view, **o** anther bottom view, **p** anther partition, **r** gynostemium

The morphological differences between the new species and the other taxa classified within the subsection *Spatellae* are presented in the Table 1.

Key to the species of *Polystachya* section *Superpositae*:

1. Flowers orange to scarlet, fleshy, almost flat when opened, lip geniculate *Polystachya kermesina*
- 1.* Flowers white, yellow, green, pink or purple, not widely opening, lip not geniculate 2
2. Inflorescence paniculate, lip shortly clawed 3
- 2.* Inflorescence simple, lip long-clawed 7
3. Flowers fairly large, mentum 4 mm long or more, lip 15–18 mm long *Polystachya stewartiana*
- 3.* Flowers small, mentum less than 4 mm long, lip 2–6 mm long 4
4. Bracts 1–2.5 mm long, mentum less than 2 mm long, rounded 5
- 4.* Bracts 3–7 mm long, mentum more than 2.5 mm long, conical 6
5. Pseudobulbs 2–4-leaved, leaves linear-lanceolate, 4–9 cm long, lip with a callus *Polystachya suerposita*
- 5.* Pseudobulbs 4–7-leaved, leaves oblong-lanceolate, 5–16 cm long, lip without a callus *Polystachya fusiformis*
6. Sepals long acuminate, lip lateral lobes oblong, middle lobe acuminate *Polystachya simplex*

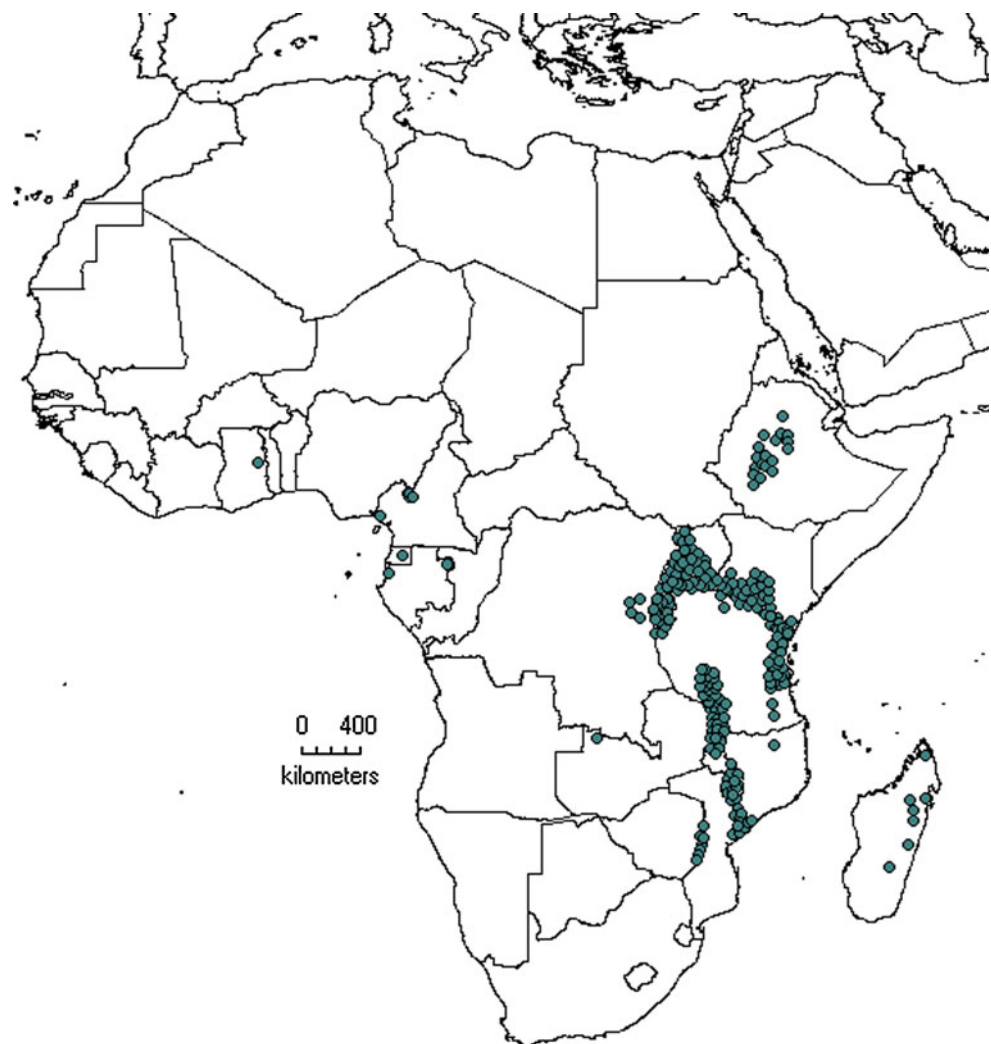
Table 1 The differences between the species of *Polystachya* Hook. section *Superpositae* Kraenzl. subsection *Spatellae* Mytnik

	<i>Polystachya aethiopica</i>	<i>Polystachya lindblomii</i>	<i>Polystachya pudorina</i>	<i>Polystachya ruwenzoriensis</i>	<i>Polystachya tridentata</i>	<i>Polystachya spatella</i>	<i>Polystachya bamendae</i>
Leaf width (mm)	6–8.5	(1)2–4	1.5–3	4–11	4–11	2.5–6	Up to 7
Pedicel and ovary	Densely pubescent	Densely pubescent	Densely pubescent	Glabrous or sparsely pubescent	Densely pubescent	Glabrous	Sparsely pubescent
Petals	Linear-lanceolate, acute	Linear to oblong-elliptic, acute	Elliptic, shortly clawed, obtuse to rounded	Spathulate, acute	Oblanceolate, acute	Oblanceolate, acute	Narrowly elliptic, acute
Lip	Obscurely 3-lobed in the middle	Obscurely 3-lobed in apical half	3-lobed in the middle	3-lobed in the middle	3-lobed in the middle	3-lobed in apical half	3-lobed in apical half
Lip claw	Glabrous	Pubescent	Glabrous or sparsely pubescent	Glabrous or sparsely pubescent	Glabrous	Densely pubescent	Densely pubescent
Lip middle lobe	Oblong, pubescent, margins irregularly toothed	Much larger than lateral lobes, broadly ovate to orbicular, apiculate	Suborbicular, margins erose, apiculate	Orbicular, acute	Orbicular-ovate, acute	Rotund-quadrate, apiculate	Transversely widely oblong, crenulate, 4-lobed, apiculate
Lip callus	Small, at base of middle lobe, fleshy	Fleshy transverse ridge between lateral lobes	Fleshy, between lateral lobes, porrect, 2–3-toothed at front	Comparatively small, centrally placed	Broad, 3-toothed, just below insertion of middle lobe and extending on the bases of lateral lobes	Obscure, fleshy, between lateral lobes	Conspicuous, fleshy oblong ridge between lateral lobes

- 6.* Sepals acute or subacute, lip lateral lobes obscure, middle lobe subacute or shortly mucronate *Polystachya eurygnatha*
7. Ovary densely pubescent 8
- 7.* Ovary glabrous or very sparsely pubescent 11
8. Leaf more than 6.5 mm wide 9
- 8.* Leaf less than 6 mm wide 10
9. Lip callus 3-toothed, lip surface glabrous *Polystachya tridentata*
- 9.* Lip callus simple, lip pubescent *Polystachya aethiopica*
10. Flowers yellow-green, petals linear *Polystachya lindblomii*
- 10.* Flowers pink or white with a crimson column, petals ovate-elliptic *Polystachya pudorina*
11. Lip middle lobe markedly longer than wide *Polystachya ruwenzoriensis*
- 11.* Lip middle lobe markedly wider than long 12
12. Mentum more than 4.5 mm long, slightly bifid, lip middle lobe oblate *Polystachya spatella*
- 12.* Mentum up to 4 mm long, deeply bilobed, lip middle lobe transversely widely oblong *Polystachya bamendae*

Polystachya bamendae, as well as *P. superposita*, are species endemic to submontane and montane forest of Cameroon (a part of Guinea Forests of West Africa Biodiversity Hotspot), (Figs. 5, 6). The representative of the newly described species was collected in the south-eastern part of the Bamenda Highlands, where the remaining patches of montane forest are very small (up to 500 ha, BirdLife International 2010), but biologically very important. As the narrowly endemic species, both *Polystachya bamendae* and *P. superposita*, should become a subject of very careful conservation action. Cameroonian Highland forests, where the species occur, are being cleared for firewood, timber and to create farmland. The Kilum-Ijim Forest, where two specimens of *P. superposita* were found, is the largest remaining patch of Afro-montane forest in West Africa being a very important area for biodiversity. Despite the same distribution range, both species are rather easy to distinguish morphologically. *Polystachya bamendae* is characterized by the fractiflex rachis, pubescent flowers, prominent deeply bilobed mentum up to 4 mm long and a fleshy clawed lip. The lip lateral lobes of the latter species are outspread, up to 2.5 mm long and 2 mm wide, the lip middle lobe is at least twice wider than long.

Fig. 5 The distribution map of *Polystachya* Hook. section *Superpositae* Kraenzl



The lip is squeezed in the middle and furnished with a prominent fleshy callus.

Discussion

Both plastid and nuclear ITS data indicate common origin of the species representing the section *Superpositae*. According to the results of the DNA analyses the most closely related species to *Polystachya bamendae* is *P. lindblomii*. The results of morphological analysis support the relation. Both species are erect or pendulous epiphytic herbs forming clumps with linear to linear-lanceolate leaves. The inflorescence of *P. bamendae* is usually longer than leaves and that of *P. lindblomii* much shorter, up to 3 cm long. Flowers of both species are more or less pubescent with prominent mentum; however, they are easily distinguishable by the lip structure. The labellum of *Polystachya bamendae* is as wide as short, the lip lateral

lobes are prominent up to 2.5 mm long and 2 mm wide, the middle lobe is transversely widely oblong, 4-lobed, emarginate with a prominent apex on the back side and the callus is fleshy bilobed ridge along the midnerve in the middle of the lip. On the contrary the lip of *Polystachya lindblomii* is twice longer than wide, the lateral lobes are small up to 0.6 mm long and 1 mm wide, the middle lobe is broadly ovate to orbicular and the lip callus is a transverse tridentate fleshy ridge.

The change of frameshift and an occurrence of the stop codon as the consequence are caused by a presence of indel 19 bp within the sequence of *matK* gene of the species of *Superpositae* (excluding *Polystachya lindblomii*) and in the newly described *P. bamendae* as well. This character is a molecular synapomorphy and along with morphological characters they prove a common origin of the representatives of *Superpositae*. Russell et al. (2010b) suggest that the *matK* gene of the mentioned taxa is a pseudogene. However, considering its function in post-transcriptional

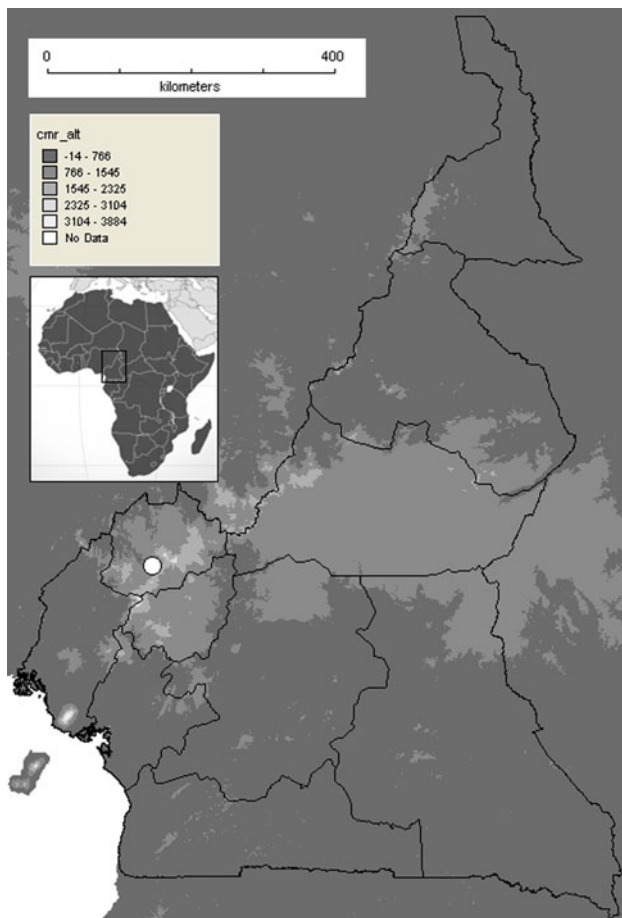


Fig. 6 The distribution map of *Polystachya bamendae* Szlachetko, Baranow & Mytnik

modification process, *matK* is essential for proper organism working. Barthet and Hilu (2007) basing on an analysis of *matK* cDNA translation did not find the stop codons in *Spathoglottis plicata* Blume (Orchidaceae), which were noted during the DNA analysis of this gene (Freudenstein et al. 2004). An analogical situation may concern the supposed pseudogene in *Superpositae*. Two alternative hypotheses supporting functionality of the *matK* gene in the mentioned species may be presented: (1) RNA editing mechanisms can improve the reading frame in species with frameshift indels and premature stop codons; (2) downstream frameshift by one base pair eliminate all stop codons in *matK* gene in all mentioned species. In this case ATA codon (for isoleucine) could be initial one. ATA and ATT (both codons for isoleucine) were reported as start codon for *Thalassiosira pseudonana* Cleve and *Phaeodactylum tricornerutum* Bohlin (Chromalveolata), respectively (Imanian et al. 2010).

The question is whether the taxa possessing the duplication are the monophyletic group? The problem is interesting, because *Polystachya lindblomii*, apart from

all other species of the section *Superpositae*, does not possess the duplication and possessing the indel seems to be a new character. If we had more samples of different representatives of *Polystachya lindblomii*, would the indel appear? We had an access to a relatively small amount of samples and therefore we are not able to unequivocally state it is a constant character, it may be a result of variability within a population or a molecular synapomorphy as well. A hypothesis is the duplication of this fragment took place in the ancestor of all species possessing today the duplication, what makes the group monophyletic under condition that *Polystachya lindblomii* is no longer classified within the group. What is surprising, the new species is the most closely related to *P. lindblomii*. The analysis of morphological characters of *P. lindblomii* and other species of the section does not support the hypothesis. To solve the problem and determine the status of indel (synapomorphy or not), an access to more samples and results of population studies are needed.

The subsectional division proposed by Mytnik-Ejsmont (2011) does not correlate with the results of the present molecular analyses. Only the nominal subsection is monophyletic, whereas representatives of the rest two subsections (*Polystachya spatella* and *P. kermesina*) form strongly supported monophyletic clade. The newly described *Polystachya bamendae* forms a weakly supporting clade with *P. lindblomii* (subsection *Spatellae*), however, in the analysis of the ITS marker only. The plastid marker does not support such relation between the two species. However, it is noteworthy that only a few species were analyzed, less than 50 percent of the representatives of the subsections *Spatellae* and *Superpositae*. Therefore, basing on such weakly representing test, there is too early in our opinion to verify the subsectional division proposed by Mytnik-Ejsmont (2011). Undoubtedly, we may state that *Polystachya lindblomii* is an isolated species within the section.

As most species of the section *Superpositae* present restricted distribution, they should be subjected to careful conservation action. These species are very fragile in their ecosystems and can easily be driven to extinction (Munishi et al. 2004). At this time we do not have enough information to discuss the conservation status of *Polystachya bamendae* due to the paucity of the population. It is known so far from a single locality in Cameroon and a field survey needs to be carried out to ascertain the distribution range of the species and propose the IUCN category.

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