

Chelystachya, a new genus of the subtribe Polystachyinae (Orchidaceae)

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Abstract: A new genus of Orchidaceae (Polystachyinae), *Chelystachya* Mytnik & Szlach. is described and illustrated basing on results of molecular and morphological studies. It is related to *Polystachya* Hook. but is distinct in having large, single-noded, spherical, succulent pseudobulbs flattened on the substratum, pendent and densely pubescent inflorescence, lowermost lip, and lip veins directed to the lip base. Results of phylogenetic analyses based on plastid markers validate the reason for a new genus. Keys for determination of all genera of the subtribe Polystachyinae and both species of *Chelystachya* are included. Two new combinations are made. A comprehensive description and full synonymy, as well as detailed ecological, phenological and distribution data and a list of 125 examined specimens are provided for each taxon treated. Because *Polystachya affinis* Lindl. is considered a holotype of *Polystachya* Hook. sect. *Affines* Kraenzl., this name is automatically a synonym of the newly introduced generic name *Chelystachya*. Other species of the former section *Affines* are classified within the section *Humiles*.

Key words: Africa, molecular phylogeny, morphology, nomenclature, *Polystachya*, *rpl16*, *rpl32-trnL*, taxonomy, *Polystachya* sect. *Humiles*

1. Introduction

The genus *Polystachya* Hook., described in 1824, received only one monographic treatment so far (Kraenzlin 1926). Since then, six sections (Summerhayes 1942, 1948; Cribb 1978) and a large number of new species have been described. Additionally, recently, some species placed in the traditionally conceived genus *Polystachya* were placed in separate genera (Mytnik-Ejsmont 2007, 2008; Mytnik-Ejsmont & Szlachetko 2007a, 2007b, 2008a, 2008b, 2011; Mytnik *et al.* 2011). That made Kraenzlin's (1926) classification unsatisfactory and out-of-date. Therefore, there is a need to revise this large and complex genus, which consists of about 230 species.

Kraenzlin (1926) established section *Affines* within the genus *Polystachya*. It includes about 40 species but it is a highly heterogeneous taxon which is difficult to circumscribe (La Croix & La Croix 1997; Davies *et al.* 2002). According to Kraenzlin (1926) and La Croix and Cribb (1998), it is characterized as follows: (1) small to medium-sized plants with clustered, ovoid to conical, usually erect but occasionally bilaterally compressed and appressed to the substrate, (2) 2-3-leaved pseudobulbs, (3) erect or rarely arcuate, racemose and often

densely pubescent (including flowers) inflorescences, (4) conspicuous bracts, fleshy, medium-sized to large for the plant, (5) usually hairy flowers with inconspicuous to somewhat prominent mentum, (6) lateral sepals free or connate only at the base, and (7) three-lobed fleshy and strongly recurved lip. Due to the high polymorphism of the section, its characterization in floras is always full of exceptions, especially in the shape of pseudobulbs, the number of stem nodes or the type of inflorescence. Extracting two species from the group, *Polystachya affinis* Lindl. and *P. bancoensis* Van der Burg, makes the section morphologically homogenous. In this paper, it is suggested that both *P. affinis* and *P. bancoensis*, reclassified into a new genus, are two very closely related species forming a group morphologically distinct from the rest of the section and from the genus *Polystachya*. Both species occur in west and west-central Africa, whereas the remaining species of the section are almost exclusively East African taxa (*P. camaridoides* Summerh., *P. obanensis* Rendle, *P. reticulata* Stévant & Droissart, and *P. testuana* Summerh. are the only exceptions, which constitute 12% of all species of the section *Affines*). The results of molecular analyses of plastid markers presented in this paper and those

recently published by Russell *et al.* (2010) confirm the legitimacy of such a treatment. Considering the morphological and molecular data we therefore suggest transferring *P. affinis* and *P. bancoensis* to a new taxon of the generic rank.

To show an isolated position of *Chelystachya affinis* and *C. bancoensis* compared to other members of the *Polystachya* genus, plastid fragments were sequenced: *rpl16* intron and *rpl32-trnL* intergenic spacer. The average length of *rpl16* in Polystachyinae is 1000 bp. Data from the *rpl16* region has been primarily used for a phylogenetic analysis at the infrageneric and familial levels (Kelchner & Clark 1997; Schnabel & Wendel 1998; Applequist & Wallace 2000). The average length of *rpl32-trnL* in Polystachyinae is 650 bp. Shaw *et al.* (2007) suggest that this is the best region of the 34 regions surveyed for low-level molecular studies. Until now, *rpl32-trnL* has not been widely used in phylogenetic studies in orchids. Barrett and Freudenstein (2009) used this marker in population study in *Corallorhiza Gagnebin*. Additionally, to increase the number of molecular characters in phylogenetic analyses, available data for the same set of samples (Russell *et al.* 2010) from plastid genome were used.

The present work is a part of a larger project on the taxonomy of the subtribe Polystachyinae Schltr. (Mytnik-Ejsmont 2007, 2008; Mytnik-Ejsmont & Szlachetko 2007a, 2007b, 2008a, 2008b, 2011; Baranow & Mytnik-Ejsmont 2009; Mytnik-Ejsmont & Baranow 2010; Mytnik-Ejsmont *et al.* 2011).

2. Materials and methods

2.1. Morphological characters

The present study was based on the examination of 125 herbarium specimens from ten herbaria: BM, BR, C, F, K, MA, MO, P, W, WAG. The standard procedure of preparing the herbarium material to facilitate stereomicroscopic observation was applied. The vegetative and generative characters of individual plants were analyzed, the flowers were taken from the middle part of the inflorescence. 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 and are available upon request. For all species and lower taxa, type material was available. The abbreviations of titles are according to those in BPH (Lawrence *et al.* 1968), BPH/S (Bridson 2004), TL2 (Stafleu & Cowan 1976-1982) and TL2/S (Stafleu & Mennega 1992), the abbreviations of the authors' names are according to Brummitt and Powell (1992).

2.2. Plant material, DNA extraction and PCR amplification and sequencing

The taxa sampling has been guided by a previous morphology-based systematic study of Polystachyinae. DNA sequences for *rpl16* intron and *rpl32-trnL* intergenic spacer were obtained from 42 and 45 taxa, respectively. Four species (*Adrorhizon purpurascens* (Thwaites) Hook.f., *Sirhookera lanceolata* (Wight) Kuntze, *Bromheadia finlaysoniana* (Lindl.) Miq., and *B. srilankensis* Kruiz. & de Vogel) were selected as outgroup taxa. Voucher information and GenBank accession numbers are provided in the Appendix.

DNA samples were obtained from the DNA Bank of the Jodrell Laboratory, Royal Botanic Gardens, Kew (<http://data.kew.org/dnabank/homepage.html>). DNA extractions were performed using a modification of the CTAB protocol (Doyle & Doyle 1987), followed by purification on caesium chloride/ethidium bromide gradients.

The *rpl16* intron and *rpl32-trnL* intergenic spacer were amplified using primers developed by Shaw *et al.* (2005, 2007 respectively). Polymerase chain reaction (PCR) amplifications were carried out in a total volume of 25 µl containing 2.5 µl 10x buffer, 1 µl 25mM MgCl₂, 1 µl 5mM dNTPs, 0.5 µl of 10µM of each primers and 1 unit of Yellow Perpetual DNA polymerase (Eux, Poland), and H₂O. The PCR cycling conditions were template denaturation at 80°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, primer annealing at 50°C for 1 min, followed by primer extension at 65°C for 4 min. The final extension step was of 5 min at 65°C. Amplified products were cleaned with the High Pure PCR Product Purification Kit (Roche Diagnostic GmbH, Germany), following the manufacturer's directions. Cycle sequencing was carried out using Big Dye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., ABI, UK) with the same primers used for PCR amplification: 2.0 µl of 5x sequencing buffer, 1 µl of Big Dye terminator with 1.5 µl of 10 µM primer (1.5 pmol), 1-2 µl of amplified product (30-90 ng/µl), and H₂O in a total of 10 µl reaction volume. Cycle sequencing conditions were as follow: 25 cycles each with 15 sec denaturation (94°C), 5 sec 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).

2.3. Sequence alignment and phylogenetic analyses

The *rpl16* intron and *rpl32-trnL* intergenic spacer were aligned by eye using SeaView v. 4 (Gouy *et al.* 2010). Matrices are available upon request from MG.

An AT-rich region of *rpl16* intron (161 bp) was excluded from the analysis because of ambiguous alignment. To increase molecular data, additional DNA sequences obtained from the same DNA sample as in our study (extraction available from RBG Kew) by Russell *et al.* 2010 were used in phylogenetic analyses. *Chelystachya bancoensis* (Van der Burg) Mytnik and Szlach. was not presented in Russell's data matrix. Maximum parsimony analysis was undertaken on the combined plastid matrix (*rpl16* intron, *rpl32-trnL* + data matrix *rps16-trnK* from Russell *et al.* 2010) 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 1000 replicates of random sequence addition. Only 10 trees were saved 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 1000 replicates and the same settings as above, except for the simple sequence addition. All characters were treated as unordered and as equally weighted (Fitch 1971).

3. Results

3.1. Molecular data

The parsimony analysis showed 54 most parsimonious trees. Statistics for one of the parsimonious is shown in Table 1. The strict consensus tree is depicted in the figure 1. The tree topology from the parsimony analysis of combined data shows the newly proposed genus, *Chelystachya*, to be monophyletic (Bootstrap support (BS) = 100%, Fig. 1) and is a sister clade to the rest of the *Polystachyinae* species (BS: 100%). A support for *Chelystachya-Polystachyinae* clade is below 50%. Going up the tree, there are several clades successively sister to the *Polystachya* s.str. clade, the grade of species-poor lineages are: *Polystachya ottoniana*, *Polystachya longiscapa*, *Dendrobianthe-Neobenthamia* clade and *Polystachya vaginata-Polystachya goetzeana* clade.

3.2. Taxonomic treatment and morphological data

Chelystachya Mytnik & Szlach., *gen. nov.* (Fig. 2)
 ≡ *Polystachya* Hook. sect. *Affines* Kraenzl., Rep. Sp. Nov. Regni Veget., Beih. **39**: 95. 1926. Generitype: *Chelystachya affinis* (Lindl.) Mytnik & Szlach. (≡ *Polystachya affinis* Lindl.).

Plantae Polystachyae affines sed pseudobulbis magnis, uninodis, sphaericis, succulentis, complanatis, ad substratum appressis, inflorescentis pendulis, dense pilosis, venis labelli basim versus differunt.

Epiphytic plants up to 50 cm long with a pendent habit. Pseudobulbs 1-5 cm in diameter, single-noded, dorsoventrally compressed, several, elliptic, subspherical or almost transversely elliptic, prostrate on the substratum, apically (1)2-3-leaved, glossy green to dull bluish green, sometimes tinged purple. Leaves 2-28 cm long, 0.7-6 cm wide, bluish green, greyish green to reddish blue with purplish midrib and margins, petiolate; petiole 0.5-14 cm long, narrow, subcylindrical, clasping the scape; blade oblanceolate, oblong-lanceolate, oblong-elliptic to obovate, acute to shortly acuminate. Inflorescence 1.5-40 cm long, laxly 1-28(60)-flowered, pendent, simple or branched, branches 1-5 cm long, peduncle covered with imbricating scarious acute sheaths, peduncle and rachis densely pubescent. Flowers fragrant, densely pubescent, the sepals yellowish green, pale brown to yellow or mustard yellow washed with green and yellow with pale red purplish markings, the petals and the lip yellow, the veins sometimes brownish purple. Floral bracts 3-14 mm long, 3-6 mm wide, ovatelanceolate to transversely ovate or ovate, long-acuminate, papery, densely pubescent. Pedicel and ovary 1.6-10 mm long, slender, densely pubescent, more densely than the sepals. Dorsal sepal 5-9 mm long, 2.7-4 mm wide, elliptic-lanceolate, oblong-elliptic to lanceolate-ovate, obtuse to subacute, 5-nerved. Lateral sepals 5-9 mm long, 4.5-7 mm wide, obliquely ovate-triangular, acute or obtuse, slightly cochleate, 4-nerved, reflexed at the apex. Mentum 3-6 mm high, saccate. Petals 4.3-7.5 mm long, 1.6-2.2 mm wide, oblanceolate, oblong-obovate to spatulate, rounded at apex, 3-nerved. Lip 5.2-8 mm long, 3-6 mm wide, strongly

Table 1. Statistics for one of the most parsimonious trees for combined plastid matrix

Matrix	Combined plastid
No. of taxa	46
Included positions in matrix	<i>rpl32-trnL</i> (1119); <i>rpl16</i> (1592) <i>rps16-trnK</i> *
Variable site	1707
Parsimony-informative sites	777
Trees (MPT)	54
Fitch tree length	2620
CI	0.76
RI	0.75
ts:tv	0.55

Explanations: CI – consistency index, RI – retention index, ts:tv – transition/transversion ratio, * - from Russell *et al.* 2010 (5340)

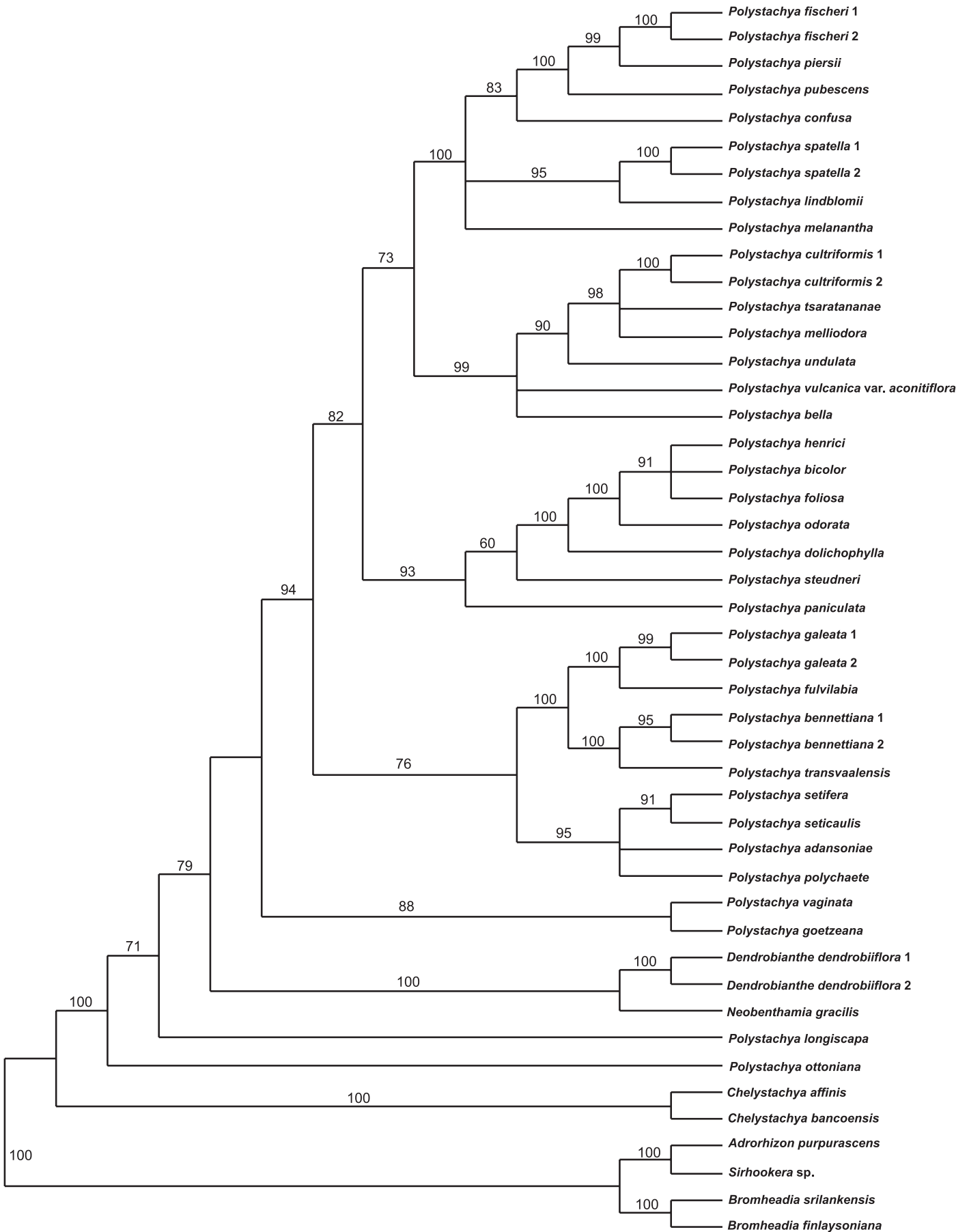


Fig. 1. The strict consensus tree of 54 most parsimonious trees from the analysis of combined plastid data for Polystachyinae. Bootstrap percentages >50 are given for supported clades above branches

to slightly recurved, three-lobed just above the middle, sometimes very obscurely pubescent at the base, lowermost due to apical drooping of the peduncle, the disc furnished with an inconspicuous central, fleshy, oblong, and pubescent callus along the mid-nerve between the lateral lobes; the lip nerves directed to its base; the middle lobe 2-3 mm long, 2-3.2 mm wide, oblong-ovate to semi-elliptic, subacute to rounded or retuse, slightly fleshy; the lateral lobes erect, narrowly oblong. Column (a free part of gynostemium) 1-2.7 mm high, semiterete, stout; column foot 2.5-8.5 mm long. Viscidium transversely narrowly-elliptic.

Etymology: The name derives from *Chelys*, the Greek word for turtle and is an allusion to the pseudobulbs looking like turtles grouped closely.

Distribution: Republic of Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Nigeria, Chad, Cameroon, Central African Republic, Equatorial Guinea, São Tomé, Gabon, Republic of the Congo, Democratic Republic of the Congo, Angola, Uganda.

Key for determination of the species. The genus comprises two species, which can be distinguishable in the following key:

1. Plant 15-50 cm long; pseudobulbs glossy green, spherical to transversely elliptic, 2.5-5 cm in diameter; lip distinctly three-lobed.....*Chelystachya affinis* 1*. Plant up to 8.5 cm long; pseudobulbs dull bluish green; elliptic to subspherical, 0.7-1.5 cm in diameter; lip obscurely three-lobed.....*Chelystachya bancoensis*

Chelystachya affinis (Lindl.) Mytnik & Szlach., **comb. nov.**
Basionym: *Polystachya affinis* Lindl., Gen. Sp. Orchid. Pl.: 73. 1830. – TYPE: SIERRA LEONE. *G. Don s.n.* (lectotype: K!; isolectotype: W!).

≡ *Polystachya bracteosa* Lindl., Edwards's Bot. Reg. 26 (Misc.): 48. 1840. ≡ *Dendrorkis bracteosa* (Lindl.) Kuntze, Revis. Gen. Pl. 2: 658. 1891. – TYPE: SÃO TOMÉ. *Masson s.n.* (holotype: K – not seen; W! – drawing in Reichenbach's collection).

≡ *Polystachya villosa* Cogn., J. Orchidées 7: 139. 1896, *hom. illeg.* (non *Polystachya villosa* Rolfe, Bull. Misc. Inform. Kew 1894: 393. 1894). ≡ *Polystachya villosula* Schltr., Beih. Bot. Centralbl. 33(2): 414. 1915. Madagascar (?), *sphalm.* – TYPE: MADAGASCAR (?). cult. Pauillac (Medoc), 'received from Linden in 1896', *M. Adde s.n.* (holotype: BR! Herb. Cogniaux).

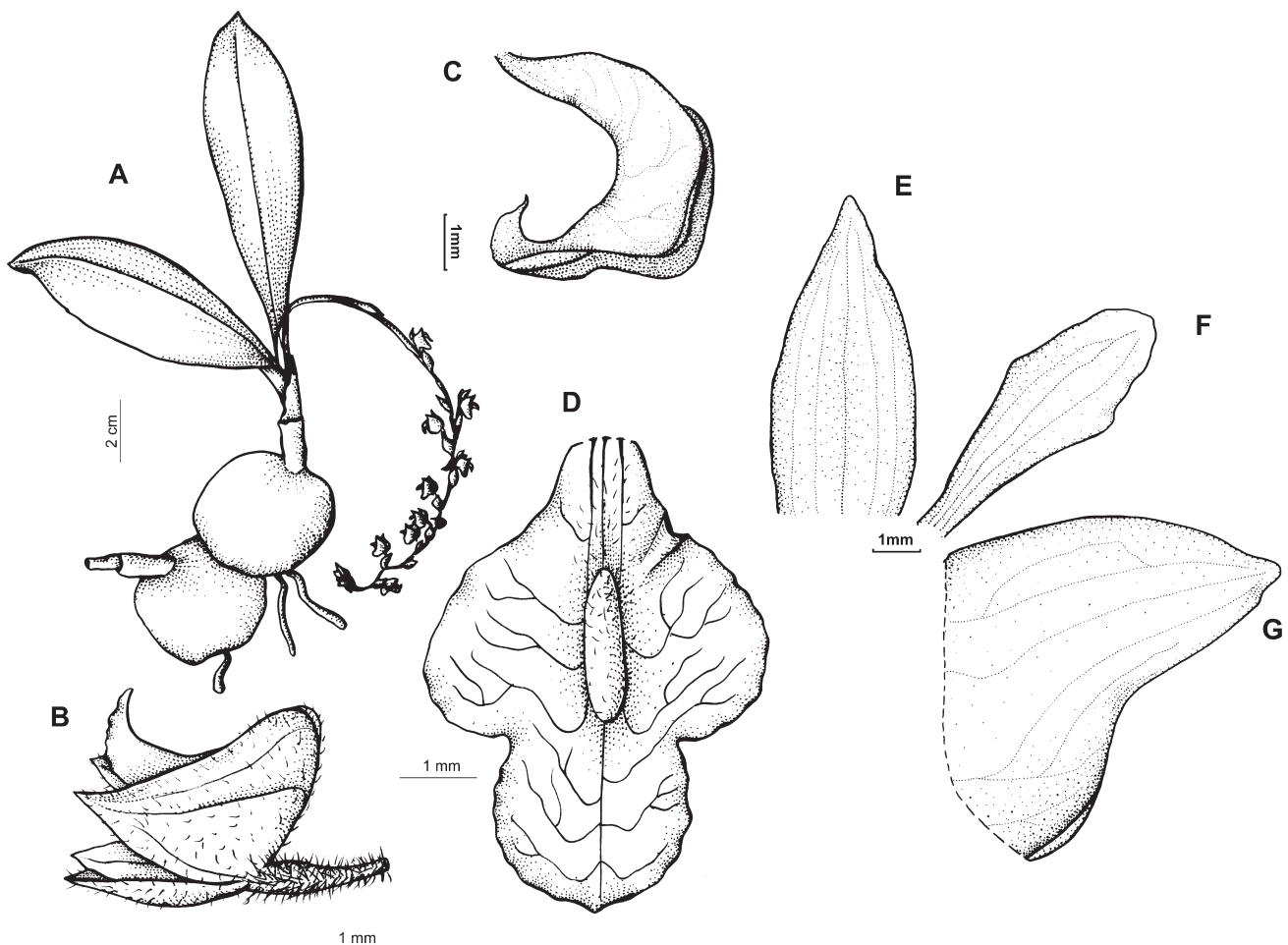


Fig. 2. *Chelystachya* Mytnik & Szlach.

Explanations: A – habit, B – flower, C – lip (in natural position), D – lip (flattened), E – dorsal sepal, F – petal, G – lateral sepal (drawn by J. Mytnik-Ejsmont from the lectotype of *Chelystachya affinis* (Lindl.) Mytnik & Szlach.)

Epiphytic plants 15-50 cm long. Pseudobulbs 2.5-5 cm in diameter, 1-noded, dorsoventrally compressed, several, subspherical, prostrate on the substratum, 2-3-leaved at apex (weak individuals 1-leaved), glossy green. Leaves 8-28 cm long, 2.6-6 cm wide, bluish green with purplish midrib and margins, petiolate; petiole 3-14 cm long, narrow, subcylindrical, clasping the scape; blade oblanceolate, oblong-lanceolate, oblong-elliptic to obovate, acute to shortly acuminate, coriaceous. Inflorescence 6-40 cm long, laxly 8-28(60)-flowered, most often pendulous, simple or branched, branches 4-5 cm long, peduncle apically drooping, covered with imbricating scarious acute sheaths, peduncle and rachis densely pubescent. Flowers medium-sized, fragrant, densely pubescent, the sepals pale brown to yellow or mustard yellow washed with green and yellow with pale red purplish markings, the petals and the lip yellow, the veins purple. Floral bracts 6-14 mm long, 5-6 mm wide, ovate-lanceolate, long-acuminate, papery, densely pubescent. Pedicel and ovary 3.5-10 mm long, slender, densely pubescent, more densely than the sepals. Dorsal sepal 5.3-9 mm long, 2.7-4 mm wide, elliptic-lanceolate, oblong-elliptic, lanceolate-ovate, obtuse to subacute, 5-nerved. Lateral sepals 6.5-9 mm long, 4.5-7 mm wide, obliquely ovate-triangular, acute or obtuse, slightly cochleate, 4-nerved, reflexed at the apex. Mentum 3.5-6 mm high, saccate. Petals 5.7-7.5 mm long, 1.6-2.2 mm wide, oblanceolate to oblong-obovate, rounded at the apex, 3-nerved. Lip 6.2-8 mm long, 4.7-6 mm wide, recurved, lowermost, obscurely three-lobed just above the middle, pubescent at the base, furnished with a not prominent central fleshy oblong and pubescent callus along the mid-nerve between the lateral lobes, merged with the disc gradually, 1.8-2 mm long; the nerves prominent, directed to the base of the lip; the middle lobe 2-3 mm long, 2.5-3.2 mm wide, oblong-ovate to semi-elliptic, subacute to rounded, slightly fleshy; the lateral lobes erect, narrowly oblong. Column 1 mm high, semiterete, stout; column foot 4-8.5 mm long. (Fig. 2)

Distribution: Republic of Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Nigeria, Chad, Cameroon, Central African Republic, Equatorial Guinea, São Tomé, Gabon, Republic of the Congo, Democratic Republic of the Congo, Angola, Uganda.

Habitat: A shade epiphyte in rain forest, riverine forest, savanna.

Flowering time: December to February.

Altitude: 50-1350 m.

Selected specimens examined: REPUBLIC OF GUINEA. Faranah: Dabola, Kouroufig, *Pobeguïn 1523* (P!); *Chevalier 20258* (BR!, P!); Sambadougou *Chevalier 20550* (K!, P!); Cerde de Faranah, Sambadougou, 26 Jan 1909, *Chevalier 20990* (K!). Labé: Pita, *Pobeguïn 2195* (P!). SIERRA LEONE.

Bombali: Karina, 10 Jan 1929, *Glanville 150* (K!); Makali, 13 Jan 1947, *Deighton 4567* (K!). Kailahun: Bumbuma, alt. 200 m, 14 Oct 1914, *Thomas 3239* (K!); *Thomas 9018* (1915), (K!); Makuta, alt. 120 m, *Thomas 483*, 7 Jun 1914, (K!); *Thomas 9018* (1915), (K!); Bunambu, in riverine forest, 28 Feb 1982, *Segeberäck 1501a* (K!); 24 May 1981, *Segeberäck 1501* (K!). Kankan: Wallia, *Scott-Elliot 4586* (P!). Kanema: near Kenema Town bridge, May 1914, *Poole 226* (K!). Port Loko: near Wallia, Jan 1930, *Scott Elliot 4627 b* (K!). Senehun: along Tibai river, Senehun (Kamjei), 16 Apr 1960, *Bakshi 128* (K!). Western Area Urban: Hastings, Feb 1956, *Gledhill 48* (K!). LIBERIA. Nimba: Ganta, 14 Feb 1951, *Harley 1772* (K!); Sanninguelle, Nimba Mts., alt. 800 m, 16 Jan 1969, *Johansson 450* (C!, K!). Grand Bassa: Herbel, behind Botanical research garden Firestone, 18 Feb 1969, *Jansen 1568* (K!). Sine loc.: alt. 500 m, 26 Jan 1965, *Adam 20761* (K!). IVORY COAST. Dix-Huit Montagnes: Tiapleu, 15 Feb 1967, *Aké Assi 9418* (K!, ABI); Mt. Tonkoui, Jan 1950, *Schnell 4082* (P!); Mt. Tonkoui, Dec 1971, *Perez-Vera 175* (ABI, P!). Bas-Sassandra: Cavally, seashore between Tabou and Béréby, 16-17 Aug 1907, *Chevalier 19967 bis* (P!); Cavally bank, Liepleu, 24 Feb 1974, *Perez-Vera 583* (K!). Worodougou: Séguéla-Mankono, 11 Mar 1974, *Perez-Vera 599* (K!). GHANA. Ashanti: Efiduase, May 1936, *Cox 31* (K!); Brafu Edru, 26 Jan 1896, *Cummins 15139* (K!). Eastern: Kwahu Nteso to Ankoma, bank of River Asuboni, 9 Jan 1968, *Bowling 36638* (K!); Tafo, cocoa farm, 5 Sep 1967, *Bowling 36628* (K!). Volta: Amedzofe, Jan 1953, *Westwood 32* (K!). NIGERIA. Ebo: Benin Prov., Sapoba Distr., 13 Nov 1948, *Keay 24666* (K!); Sapoba Forest Reserve, fresh water swamp forest on banks of Jamieson River, 15 Jan 1959, *Keay 37735* (K!); Benin Prov., Iyekuselu, 6 Dec 1961, *Daramola 45653* (K!). Ogun: near Ogere and Abeokuta, 28 Jan 1945, *Burt 27* (K!). Olibo: S Nigeria, Dagea Distr., 1916, *Talbot 3776* (K!). Ondo: Owo Distr., Owo, Sep 1948, *Keay 22714* (K!); *Keay 22714* (K!); Akure Distr., Akure Ofosu, 4 Dec 1961, *Adebuayui 4531 (45324?)* (K!). Plateau Province: Kwarra, Proiunca (?), *King 538* (K!). CHAD. Haut Oubangui: Krebedje (Fort Sibut), *Chevalier 10780* (P!); Bangui *Chevalier 10837* (P!); *Chevalier 10914* (P!); *Chevalier s.n.* (P!). CAMEROON. South: Bitye pres riv. Dja, 50 km ENE Sangmelima, 28 Dec 1921, *Bates 1886* (K!); Bitye, near the River Dja *Bates s.n.* (P!); Bella, 45 km NE Kribi, 24 Jan 1962, *Letouzey 4122* (K!, P!); 10 km N of Kribi *Raynal 13497* (P!); Embouchure de l'estuaire de la Lobe, S Kribi, 10 Feb 1969, *Sanford 5701* (K!, P!); Kribi, 1912, *Zenker 4382* (BM!, K!); Nkomo pres Ngoase, S riv. Lobe, 14 Feb 1962, *Letouzey 4235* (K!, P!); Lokundje, Feb 1911, *Zenker 4* (F!). East: Entre Alat Makay et riv. Djable, 20 km NNE Mintom II, 4 Jan. 1973, *Letouzey 11783* (K!, P!). Littoral: Douala-Edea

Forest, 1978, *Thomas 206* (K!). Southwest: alt. 50 m, 30 Oct 1985 *Thomas 4911* (MO!). Centre: Bord de la riv. Soo, 78 km S de Yaounde *Villiers 1107* (P!). Sine loc.: *Bates 1254* (BM!); *Bates 1265* (BM!). CENTRAL AFRICAN REPUBLIC. Haute-Kotto: Yalinga, 16 Oct 1922, *Le Testu 4221* (BM!); Yalinga, 16 Oct 1922, *Le Testu 4228* (BM!, BR!, K!). Ouaka: Rocher chutes Gumburu, Wamire, 60 km N Bambari, 27 Nov 1928, *Tisserant 2063* (BM!). EQUATORIAL GUINEA. Rio Muni: Nkolendangen, alt. 450 m, 8 Jan 1908, *Tessmann 99* (K!); Bata-Sendje (Senye), estrada km 40, a jussante da ponte sobre Rio Benito, bosque primario, 30 May 1993, *Carvalho 5320* (MA!); Bata-Bicomo, en las margenas del Rio Ecucu, al lado de la central hidroelectrica, bosque primario, 30 Jun 1997, *Carvalho 6377* (MA!). Pagalu: Mt. Cacinca, S of San Pedro, alt. 250 m, 21 Jul 1959, *Melville 176* (K!). SÃO TOMÉ. Sine loc.: *Masson s.n.* (W!). GABON. Ngounie: 2 km on road from Lebamba to Mbigou, 2°13'S, 11°29'E *De Wilde & Sosef 10418* (WAG!). Ogooue-Ivindo: Station d'Ipassa, 10 km S de Makokou, Nyame Pende, *Florence 1122*, (P!*-p.p.*); Ayem, 10 km SW de Ndjole *Hallé 1965* (P!); Francesville, *Thollon 816* (P!). Haute-Ngounye: bord de la Ngounye, Litsila, Moupoude, 20 Jun 1927, *Le Testu 6511* (BM!, K!, P!); Ngwa *Le Testu 9174* (BM!, P!). Estuaire: ca. 30 km E of Cocobeach, Ekorodo-village, 0°56'N, 9°57'E, *Reitsma 2312* (WAG!). Sine loc.: 12 Apr 1920, *Le Testu 8017* (BM!). REPUBLIC OF THE CONGO. Likouala: Impfondo, *Chevalier 10961* (P!); Impfondo, *Chevalier 11011 bis* (P); Komi, *Lecomte s.n.* (P!). Bouenza: Mouyondzi *Farron 4599* (P!). DEMOCRATIC REPUBLIC OF THE CONGO. Bas-Congo: Kinshasa, *Bequaert 8039* (BR!); Zongo, *Breyne 149* (BR!); Zundu, *Compere 1684* (BR!); Kisantu, *Gillet s.n.* (BR). Kasai: Tshimboa, *Callewaert s.n.* (BR!). Forestier Central: Dundusana, *Mortehan 987* (BR!); Ngazi Riv., Aruwimi, *Louis 12180* (BR!); Yangambi, *Louis 6318* (BR); Irebu, *Laurent 1* (BR!); Likako, *Evrard 3040* (BR!); lac Tumba, *Laurent s.n.* (BR!). Ubangi-Uele: Suronga, *Seret 155 bis* (BR!); route Bima-Bambili, *Seret 155* (BR!). ANGOLA. Lunda Sul: Lunda, Dundo, Rio Lunachi, 18 Apr 1937, *Carrisso & Sousa s.n.* (BM!); River Luembe near Cossa, alt. 700 m, 12 Jun 1948, *Gossweiler 14077* (BM!, K!). UGANDA. Mengo Distr.: Mpanga Forest, Feb 1965, *Karnya in Leakey s.n.* (K!). Nomenclatural Notes. *Polystachya villosa* was described from a plant cultivated by M. Adde from Pauillac, who "received it from Madagascar" (according to Perrier 1936). It has not been rediscovered again and the provenance of the material remains doubtful. Moreover, *Polystachya villosula* was merely a *sphalm* for *P. villosa* Cogn. (Kraenzlin 1926).

Chelystachya bancoensis (Van der Burg) Mytnik & Szlach., *comb. nov.*

Basionym: *Polystachya bancoensis* Van der Burg, Misc. Papers Agric. Univ. Wag. **19**: 23. 1980. ≡ *Polystachya affinis* var. *nana* J.B.Hall, Kew Bull. **29**(2): 428. 1974. – TYPE: GHANA. Western region: Tanoso, *Bowling in GC38171* (Holotype: K!; Isotype: GC – not seen, Photo: WAG!).

Dwarf epiphytic plants up to 8.5 cm long. Pseudobulbs 1-2.5 cm long, 0.7-1.5 cm in diameter, dorsoventrally compressed, subspherical to elliptic, prostrate on the substratum, 1-2-leaved at apex, dull bluish green, sometimes tinged purple. Leaves 2-6 cm long, 0.7-1.5 cm wide, bluish green, greyish green to reddish blue with purplish midrib and margins, thin-textured, petiolate; petiole up to 1 cm long, narrow, subcylindrical; blade oblanceolate, lanceolate-elliptic to lanceolate-ovate, acute to shortly acuminate. Inflorescence 1.5-7 cm long, laxly 1-6(13)-flowered, pendent, simple, sometimes branching, branches 1-2, short; peduncle with 1-2 bracts, apically drooping, rachis flexuous, peduncle and rachis pubescent. Flowers rather small, minutely and densely pubescent outside, yellowish green with pale red purplish markings, the pedicel and ovary purplish red, the base of the anther and the base of the column reddish, and sometimes the lip nerves brownish. Floral bracts 3-5 mm long, 3 mm wide, suborbicular, ovate to transversely ovate, acuminate to tridentate, papery, densely pubescent. Pedicel and ovary 1.6-4 mm long, slender, densely hairy, hairs up to 0.4 mm long. Dorsal sepal 5-6.7 mm long, 3-4 mm wide, elliptic-ovate or oblong-elliptic, obtuse. Lateral sepals 5-6 mm long, obliquely elliptic- to triangular-ovate, subacute or subobtuse, slightly cochleate. Mentum 3-4 mm long, saccate. Petals 4.3-5.7 mm long, 2 mm wide, obovate to spatulate, rounded at apex, glabrous. Lip 5.2-7.3 mm long, 3-3.8 mm wide, obscurely three-lobed, lowermost, fleshy along the mid-nerve, slightly recurved, elliptic to oblong-elliptic in general outline, furnished with an oblong inconspicuous callus in the basal half; the middle lobe 2-3 mm long, 2-2.4 mm wide, semi-elliptic, retuse to round at the apex, slightly fleshy; the lateral lobes 1-2.2 mm long, porrect, transversely semi-elliptic. Column 2.7 mm high, column foot 3-3.5 mm long, viscidium transversely narrowly-elliptic.

Distribution: Liberia, Ivory Coast, Ghana.

Habitat: A fully exposed trunk epiphyte on high branches, in humid evergreen forest. Flowering time. November.

Altitude: 0-400 m.

Representative Specimens Examined: LIBERIA. Sine loc.: cult., *De Wit s.n.* (WAG!). IVORY COAST. Agnéby: Yapo, 3 Nov 1974, *Perez-Vera 722* (K!, P!). Bas-Sassandra: San Pedro, km 14 road Grand-Bereby-Tabou, 4°43'N, 7°00'W, *Kruif 709* (WAG!). Dix-Huit Montagnes: Tiapleu, Nov 1966, *Ake Assi 9228* (K!); Téké, 22 Nov 1966, *Ake Assi 9308* (K!). Haut-

Sassandra: Mt. Mafa, 28 Oct 1965, *Bonardi s.n.* (P!). Lagunes: Banco Forest Reserve, 22 Oct 1975, *van der Burg 1245* (WAG!); Banco Forest Reserve, near entrance, 10 Dec 1974, *de Koning 5005* (WAG!); Adiopodoumé, 4°08'W, 5°20'N, *Dekker 328* (WAG!); Adiopodoumé *Perez-Vera 152* (K!, P!); Anguededou, 7 Jan 1975, *de Koning 5120 A* (WAG!). Sine loc.: *van der Burg 1269* (WAG); *de Koning 6102* (WAG!); *de Koning 6107* (WAG!); *de Koning 6115* (WAG!); *de Koning 6116* (WAG!). GHANA. Western region: Tanoso, *Bowling in GC 38171* (K!).

4. Discussion

4.1. Phylogenetic relationships

The tree obtained in this study (Fig. 1) provides a valuable resolution at the subtribal level, permitting the comparison of the molecular phylogenetic framework with the traditional classification of *Polystachya*. The results of recent molecular studies conducted by Russell *et al.* (2010) and our morphological and molecular analyses presented here confirm isolated positions of some taxa being classified within *Polystachya* till now.

The first clade comprises the complex of two species: *Chelystachya affinis* (classified as *Polystachya affinis*) and *Chelystachya bancoensis* (classified as *Polystachya bancoensis*). The tree indicates the need to distinguish the *P. affinis*-group at the generic rank and morphological characters also confirm the status change (see below for detailed discussion). Thus, the newly proposed genus is shown to be monophyletic. The monophyly of the clade formed by the two species of the new genus is well supported.

Another taxon that deserved to be placed in a separate genus is *Polystachya longiscapa* Summerh., a member of 'grade of species-poor lineage, as it was defined by Russell *et al.* (2010). The results obtained by these authors and our phylogenetic tree show the taxon occupies an isolated position within Polystachyinae. The problem with the classification of *P. longiscapa* was presented by many authors, e.g. Summerhayes (1934), Cribb (1984) and Cribb and King (2006). Summerhayes (1934) observed that *P. longiscapa* approaches the remarkable monotypic genus *Neobenthamia* Rolfe, Cribb (1984) classified the species within the section *Dendrobianthe* (Schltr.), Mytnik, Cribb and King (2006) noticed the species occupies rather an isolated position within *Polystachya*. In our opinion, *P. longiscapa* occupies an intermediate position between *Dendrobianthe* and *Neobenthamia* and detailed morphological studies and the results of Russell *et al.* (2010) confirm the taxon deserves a generic rank. In 2011, *Polystachya longiscapa* was placed in a separate monotypic genus *Neoburtia* (Mytnik *et al.* 2011).

Neobenthamia, representing the next lineage, is a well defined monotypic genus closely related to

Dendrobianthe and "*P. longiscapa* clade" (*Neoburtia*) and we disagree with Russell *et al.* (2010) that it should be included within *Polystachya*. *Neobenthamia* is sister to *Dendrobianthe dendrobiiflora* (Rchb.f.) Mytnik but the former genus differs from *Polystachya* in many aspects (e.g. lack of pseudobulbs, lowermost lip, obscure column foot and mentum, pollinia subsessile on the viscidium) and in our opinion they are two distinct genera. Including *Neobenthamia* within *Polystachya* makes the latter genus heterogenic and difficult to define.

Dendrobianthe dendrobiiflora is a species representing one of recently described genera and in the present analysis forms another well supported clade. *Dendrobianthe*, excluding *Dendrobianthe longiscapa*, comprises three species [*D. dendrobiiflora*, *D. bequaertii* (Summerh.) Mytnik, and *D. zuluensis* (L. Bolus) Mytnik] clearly differing from *Polystachya* s.str. by e.g. slender stems thickened into pseudobulbs at the base only, narrow, grass-like leaves predominantly absent at the flowering time, widely open flowers, unlobed or rarely very obscurely trilobed lip with a central pubescent thickening.

Polystachya goetzeana (Kraenzl.) Mytnik & Szlach. and *P. vaginata* (Summerh.) Mytnik & Szlach. form the last lineage of the base of the tree and the clade is sister to *Polystachya* s. s. The results of both molecular analyses (Russell *et al.* 2010 and that presented here) show the species, being representative of the section *Isochiloides* Summerh., occupy an isolated position in the tree and it was distinguished as another taxon of generic rank within Polystachyinae *Isochilostachya* Mytnik & Szlachetko (2011). From closely related *Polystachya*, the species of *Polystachya goetzeana-vaginata* clade differ in the structure of foliage, shape and size of leaves and floral bracts, form and length of sepals, lip pubescence and the gynostemium length.

Molecular analyses revealed a striking position of *P. ottoniana* Rchb.f., the clade being sister to *Neobenthamia*, *Dendrobianthe*, *Polystachya* section *Isochiloides* and *Polystachya* s.str. clades. The position of *P. ottoniana*, a representative of the section *Affines* Kraenzl., is difficult to explain from a morphological point of view. We examined over forty specimens of the species in details and we cannot find any explanation for such position of *P. ottoniana* in the tree. In our opinion further detailed studies are needed to resolve the problem.

To sum up, we found that *Polystachya* s.l., including *Neobenthamia*, newly proposed *Chelystachya*, *Neoburtia* (*P. longiscapa*), *Dendrobianthe*, *Isochilostachya* (*P. vaginata*-*P. goetzeana* clade) and other five recently described genera, not present in our analysis because of lack of DNA material [*Disperanthoceros* Mytnik & Szlach., *Epiphorella* Mytnik & Szlach., *Geerinkia* Mytnik & Szlach., *Szlachetkoella* Mytnik, and *Unguiculabia* Mytnik & Szlach.], is a heterogeneous taxon, difficult to define. Thus we recognize *Polystachya* in a narrow

sense. Differences between the genera are presented in the key.

4.2. Morphological differences between *Polystachya* and *Chelystachya*

The representatives of the newly proposed genus *Chelystachya* are clearly distinct from all the other representatives of *Polystachya*. They are small to medium-size plants (up to 50 cm long) with a unique hanging habit (pendent inflorescence) and very characteristic flattened, spherical, and 1-noded pseudobulbs with an apical pendent and densely pubescent inflorescence. The lip is lowermost due to the drooping inflorescence. Such exposure emphasizes colours and patterns to attract pollinators and facilitate pollination (Ernst & Arditti 1994). The other distinctive feature of *Chelystachya* is the venation pattern on the lip. The veins are directed toward the lip basal half, not apical part, as in *Polystachya*.

The species of the *Chelystachya* are exclusively trunk epiphytes in riverine or rain forests in Africa. They grow on high, mossy or naked branches. They can be found in relatively cool climates where there is high humidity throughout the year. The *Chelystachya* species grow at sea level up to 1,350 meters a. s. l. and flower from November till February (Hall 1974).

Unlike *Chelystachya*, the *Polystachya* plants are erect plants of various sizes (from 1 cm to more than 1 m high). *Polystachya* are mainly epiphytic but sometimes also lithophytic or terrestrial plants. Their stems are often thickened at the basal part or the stems are almost entirely thickened. Pseudobulbs are most often narrowly to widely elliptic to cylindrical or conical, laterally compressed, not flattened on the substratum as in *Chelystachya*. The other characters setting *Polystachya* apart from *Chelystachya* are erect to semi-erect inflorescences, flowers with an uppermost lip and the lip venation directed to the apical part of the lip.

The main differences between *Chelystachya* and *Polystachya* are presented in Table 2.

4.3. Key for determination of Polystachyinae

The subtribe *Polystachyinae* presently includes ten genera and they are keyed as follows:

Key for determination of the genera of Polystachyinae Schltr.:

- 1. Leaves absent at the anthesis.....2
- 1*. Leaves present at the anthesis.....3
- 2. Lip variously lobed, furnished with a prominent cushion-like crest in the middle, deflexed just above the base, with elevated keel present at lip base.....*Epiphorella* Mytnik & Szlach.
- 2*. Lip entire, with a single callus, if any, not pulvinate*Dendrobianthe* Mytnik
- 3. Robust plants 50-120 cm tall with pseudobulbs, sepals 16-20 mm long, mentum 5-6.5 mm long..... 12.3.*Neoburtia* Mytnik, Szlach. & Baranow
- 3*. Not as above.....4
- 4. Inflorescence pendent or apically drooped, lip of lowermost position.....5
- 4*. Inflorescence erect to semi-erect, lip of uppermost position.....6
- 5. Pseudobulbs large, spherical, succulent, flattened on the substratum, inflorescence pendent.....*Chelystachya*, gen. nov.
- 5*. Pseudobulbs absent, stem reed-like, inflorescence erect with a rachis apically drooped...*Neobenthamia* Rolfe
- 6. Spur very long and narrow, three times longer than the dorsal sepal.....*Disperanthoceros* Mytnik & Szlach.
- 6*. Spur (mentum) up to two times longer than the dorsal sepal.....7
- 7. Whole plant pendent or creeping, leaves fleshy, imbricate, bilaterally flattened.....*Szlachetkoella* Mytnik
- 7*. Plant neither pendent nor creeping, leaves not imbricate.....8
- 8. Lip with a long claw, at least third of the lip length, lip divided into 3 parts with the mesochile variously lobed, mentum elongate, saccate, swollen at the apex*Unguiculabia* Mytnik & Szlach.
- 8*. Lip shortly clawed or sessile, usually 3-lobed, then divided into the lateral and middle lobes, mentum conical-saccate, obtuse.....9
- 9. Leaves elliptic, ovate or obovate, lip often furnished with pseudopollen, sepals up to 8 mm long, obtuse, column part short and massive, 1-2.5 mm long.....*Polystachya* Hook.
- 9*. Leaves narrow, grass-like, lip without pseudopollen, covered with numerous clavate or capitate prominent, long hairs, sepals more than 8.5 mm long, acuminate, column part slender, 2-4 mm long.....*Isochilostachya* Mytnik & Szlach.

Table 2. Morphological differences between *Polystachya* Hook. and *Chelystachya* Mytnik & Szlach.

	<i>Polystachya</i>	<i>Chelystachya</i>
Pseudobulbs	narrowly to widely elliptic or conical or even inconspicuous, laterally compressed, not flattened on the substratum	almost spherical, dorsoventrally compressed, flattened on the substratum
Inflorescence	erect to semi-erect	pendent
Lip	uppermost	lowermost
Lip venation	directed to the apical part of the lip	directed to the lip base

4.4. The species of *Chelystachya*

Chelystachya bancoensis and *C. affinis* are two closely related species. The differences between them were presented by Hall (1974), who described a 'depauperate form' of *Polystachya affinis* (today's *Chelystachya affinis*) in a varietal rank as *P. affinis* Lindl. var. *nana*. Six years later, Van der Burg (in Arends *et al.* 1980) raised the variety to the species rank describing it as *P. bancoensis*. Until 1974, *Chelystachya bancoensis* was considered to be a smaller form of *C. affinis*. But the observations by Hall (1974) and Arends *et al.* (1980), during a several year cultivation period, proved the species has retained its distinctive features and no intermediate forms have been found. Both species differ in many ways, especially, in the size of plants and floral bracts and the shape and pubescence of the lip. The main morphological differences are presented in Table 3. According to Hall (1974) and Arends *et al.* (1980), there are also ecological and phenological differences: *C. affinis* is a shade epiphyte found in very damp areas. It grows on mossy branches, where the climate is relatively cool with high humidity throughout the year and reduced solar radiation because of mist and clouds. The species flowers one or two months after *C. bancoensis* (Arends *et al.* 1980), from late December to February, though, this difference can be caused by ecological differences. *C. bancoensis* grows fully exposed on the bare bark of high branches in the evergreen forest. It grows at lower altitudes, in a climate with a dry season. *C. bancoensis* can be deciduous; during the dry season it may drop its rather fragile leaves. It flowers in November (Hall 1974).

4.5. Taxonomic implications

Because *Polystachya affinis*, the type of *Polystachya* sect. *Affines*, is now transferred to the newly established

genus *Chelystachya*, this makes automatically this sectional name a nomenclatural synonym of this new generic name. Consequently, species that are still residual in the genus *Polystachya* constitute a separate section. Thus, we propose to include them in the formerly existing section *Humiles*, described by Summerhayes (1942). *Humiles* includes the vast majority of the species formerly classified within the section *Affines* sensu Kraenzlin (1926).

Polystachya Hook. sect. *Humiles* Summerh.

Bot. Mus. Leafl. [Harv. Univ.] 10: 284. 1942. – TYPE SPECIES: *Polystachya ottoniana* Rchb.f.

Summing up, the genus *Polystachya* section *Humiles* comprises 39 species: *Polystachya acridolens* Summerh., *P. acuminata* Summerh., *P. bella* Summerh., *P. bruechertiae* Eb.Fisch., Killmann & J.-P.Lebel, *P. camaridioides* Summerh., *P. campyloglossa* Rolfe, *P. confusa* Rolfe, *P. disiformis* P.J.Cribb, *P. epiphytica* De Wild., *P. fischeri* Rchb.f. ex Kraenzl., *P. haroldiana* Rolfe, *P. heckmanniana* Kraenzl., *P. holmesiana* P.J. Cribb, *P. holstii* Kraenzl., *P. johnstonii* Rolfe, *Polystachya kaluluensis* P.J. Cribb & la Croix, *P. laurentii* De Wild., *P. lawrenceana* Kraenzl., *P. leucosepala* P.J. Cribb, *P. mafingensis* P.J.Cribb, *P. magnibracteata* P.J.Cribb, *P. malilaensis* Schltr., *P. melanantha* Schltr., *P. mzuzuensis* P.J.Cribb & la Croix, *P. ngomensis* McDonald & McMurtry, *P. obanensis* Rendle, *P. ottoniana* Rchb.f., *P. piersii* P.J.Cribb, *P. praecipitis* Summerh., *P. proterantha* P.J.Cribb, *P. pubescens* (Lindl.) Rchb.f., *P. purpleobracteata* P.J.Cribb & la Croix, *P. reticulata* Stévant & Droissart, *P. sandersonii* Harv., *P. teitensis* P.J.Cribb, *P. testuana* Summerh., *P. valentina* la Croix & P.J.Cribb, *P. villosa* Rolfe and *P. zambesiaca* Rolfe.

Table 3. Morphological differences between *Chelystachya affinis* (Lindl.) Mytnik & Szlach. and *C. bancoensis* (Van der Burg) Mytnik & Szlach.

	<i>C. affinis</i>	<i>C. bancoensis</i>
Plant size	up to 50 cm	up to 8.5 cm
Pseudobulbs	mostly circular to almost transversely elliptic glossy green	usually elliptic dull bluish green, sometimes tinged purple
Flowers	opened	do not open as much as in <i>C. affinis</i>
Flower colour	brownish-yellow	yellowish-green
Floral bracts	6-14 mm long reach at least half-way up the dorsal sepal	3-5 mm long short, hardly longer than the ovary
Sepals and lip	markedly curved backwards in the upper part	almost straight
Lip	with conspicuous deep red veins	occasionally with some faint reddish lines
Callus	finishes abruptly	merges with the lip gradually
Hairs on the lip	confined to the median zone	near the margins on the upper side of lip

Acknowledgments. We wish to thank the curators and staff of the cited herbaria for their kind hospitality and assistance during our visits (BM, BR, C, K, MA, P, W, WAG) and for providing the material available on loan (MO, F). We would also like to express our gratitude to Dr. Guy Chiron, the chief editor of *Richardiana*, for his priceless help in translating the

diagnosis of the new genus. Our special thanks go to Dr. Przemyslaw Baranow for critical review of the manuscript. We are also obliged to Anton Russell for an access to his DNA sequences and stimulating discussions. This study was supported by a grant from the Ministry of Science and Higher Education (N N303 343735).

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Appendix. Voucher information and GenBank accession numbers for samples used in this study

Taxon	Voucher	GenBank No. <i>rpl16</i>	GenBank No. <i>rpl32- trnL</i>	GenBank No. <i>rps16- trnK</i> *
<i>Adrorhizon purpurascens</i> (Thwaites) Hook.f.	Kew DNA 15745, Chase 15745 (K)	HQ222153	HQ222257	GQ145084
<i>Bromheadia finlaysonian</i> (Lindl.) Miq.	Kew DNA 21766, Duangjai 039 (BRUN, K)	HQ222154	HQ222254	GQ145085
<i>Bromheadia srilankensis</i> Kruij. & de Vogel	Kew DNA 15745, Chase 15745 (K)	HQ222155	HQ222253	GQ145086
<i>Chelystachya affinis</i> (Lindl.) Mytnik and Szlach. (2)	Kew DNA 21165, Chase 21165 (K)	HQ222149	HQ222250	GQ145090
comb. nov. <i>Chelystachya bancoensis</i> (Van der Burg) Mytnik and Szlach. comb. nov.	Heidelberg BG 120907	HQ222150	HQ222251	-
<i>Dendrobianthe dendrobiiflora</i> (Rchb.f.) Mytnik (1)	Kew DNA 17962, PCP 063/98/1621 (EA)	HQ222146	HQ222243	GQ145126
<i>Dendrobianthe dendrobiiflora</i> (Rchb.f.) Mytnik (2)	Kew DNA 19184, Mugambi & Odhiambo 064/98/1622 (EA)	HQ222147	HQ222242	GQ145127
<i>Neobenthamia gracilis</i> Rolfe (1)	Kew DNA O-340, Carlswald 311	HQ222148	HQ222240	-
<i>Neobenthamia gracilis</i> Rolfe (1)	HBV ORCH07214	-	-	GQ145087
<i>Polystachya adansoniae</i> Rchb.f.	Kew DNA 17957, B 429/94/469 (EA)	HQ222138	HQ222160	GQ145088
<i>Polystachya bella</i> Summerh.	Kew DNA 17950, B & N 783 (EA)	-	HQ222233	GQ145095
<i>Polystachya bennettiana</i> Rchb.f. (1)	Kew DNA 19186, Mugambi & Odhiambo 81/01 (EA)	HQ222141	HQ222194	GQ145097
<i>Polystachya bennettiana</i> Rchb.f. (2)	Kew DNA 17958, B 338/94/418 (EA)	HQ222140	HQ222193	GQ145096
<i>Polystachya bicolor</i> Rolfe (2)	Kew DNA 25884, Kew living collection 2003-406 (WU)	HQ222121	HQ222212	GQ145120
<i>Polystachya confusa</i> Rolfe	Kew DNA 17947, B <i>et al.</i> 122 (EA)	HQ222101	HQ222173	GQ145121
<i>Polystachya cultriformis</i> (Thouars) Sprengel (1)	Kew DNA 19183, Mugambi & Odhiambo 054/98/1612	HQ222109	HQ222190	-
<i>Polystachya cultriformis</i> (Thouars) Sprengel (2)	Kew DNA 19182, Mugambi & Odhiambo 054/98/1607 (EA)	HQ222133	HQ222189	GQ145124
<i>Polystachya dolichophylla</i> Schltr.	Kew DNA 25886, Chase 25886 (K)	GU045350 ^a	HQ222170	GQ145128
<i>Polystachya fischeri</i> Rchb.f. ex Kraenzl. (1)	Kew DNA 17964, Pearce 616/94/607 (EA)	HQ222116	HQ222180	GQ145133
<i>Polystachya fischeri</i> Rchb.f. ex Kraenzl. (2)	Kew DNA 17965, B 674/95/1280 (EA)	HQ222115	HQ222177	GQ145134
<i>Polystachya foliosa</i> (Hook.) Rchb.f.	Kew DNA 25887, Kew living collection 2001-3986	GU045351 ^a	HQ222205	GQ145135
<i>Polystachya fulvilabia</i> Schltr.	Kew DNA 17855, Chase 17855 (K)	HQ222105	HQ222161	GQ145137
<i>Polystachya galeata</i> (Sw.) Rchb.f. (1)	Kew DNA O-1496, Chase O-1496 (K)	HQ222108	HQ222216	GQ145139
<i>Polystachya galeata</i> (Sw.) Rchb.f. (2)	Kew DNA 14650, Chase 14650 (K)	HQ222117	HQ222220	GQ145140
<i>Polystachya goetzeana</i> Kraenzl.	Kew DNA 17955, B & L 1772 (EA)	-	HQ222252	GQ145141
<i>Polystachya henrici</i> Schltr.	Kew DNA 17856, Chase 17856 (K)	HQ222128	HQ222211	GQ145145
<i>Polystachya lindblomii</i> Schltr.	Kew DNA 17967, B 1142/98/1695 (EA)	HQ222134	HQ222174	GQ145154
<i>Polystachya longiscapa</i> Summerh.	HBV ORCH06411	-	-	GQ145155
<i>Polystachya melanantha</i> Schltr.	Kew DNA 17954, B & L 1783 (EA)	HQ222135	HQ222179	GQ145157
<i>Polystachya melliadora</i> P. J. Cribb	Kew DNA 17923, Chase 17923 (K)	HQ222124	HQ222197	GQ145158
<i>Polystachya odorata</i> Lindl.	Kew DNA 17857, Chase 17857 (K)	GU045360 ^a	HQ222215	GQ145164
<i>Polystachya ottoniana</i> Rchb.f.	Kew DNA 25888, Kew living collection 2005-964	-	HQ222248	GQ145168
<i>Polystachya paniculata</i> (Sw.) Rolfe	Kew DNA 25889, Kew living collection 1984-4977	HQ222137	HQ222207	GQ145170
<i>Polystachya piersii</i> P. J. Cribb	Kew DNA 17948, B <i>et al.</i> 101/95/1186 (EA)	HQ222125	HQ222178	GQ145172/
<i>Polystachya polychaete</i> Kraenzl.	Kew DNA 25890, Kew living collection 2001-3987	HQ222118	HQ222224	GQ145178
<i>Polystachya pubescens</i> (Lindl.) Rchb.f.	Kew DNA O-700, Kurzweil 1849	HQ222103	HQ222183	GQ145179
<i>Polystachya seticaulis</i> Rendle	Kew DNA 17924, Chase 17924 (K)	HQ222099	HQ222168	GQ145186
<i>Polystachya setifera</i> Lindl.	Kew DNA O-1493, Chase O-1493 (K)	HQ222111	HQ222237	GQ145187
<i>Polystachya spatella</i> Kraenzl. (1)	Kew DNA 17951, B 949 (EA)	HQ222104	HQ222184	GQ145188
<i>Polystachya spatella</i> Kraenzl. (2)	Kew DNA 19263, Khayota 381 (EA)	HQ222102	HQ222185	GQ145189
<i>Polystachya steudneri</i> Rchb.f.	Kew DNA bank 17956, B 712/95/1305 (EA)	HQ222119	HQ222169	GQ145190
<i>Polystachya transvaalensis</i> Schltr.	Kew DNA 19264, Bytebier & Luke 1774 (EA)	HQ222126	HQ222191	GQ145197
<i>Polystachya tsaratanananae</i> H. Perrier	Kew DNA 17861, Chase 17861 (K)	HQ222100	HQ222196	GQ145199
<i>Polystachya undulata</i> P. J. Cribb & A. Podzorski	Kew DNA 17862, Chase 17862 (K)	HQ222123	HQ222208	GQ145203
<i>Polystachya vaginata</i> Summerh.	Kew DNA 17949, B 566/95/1140 (EA)	HQ222113	HQ222245	GQ145204
<i>Polystachya vulcanica</i> Kraenzl. var. <i>acotiflora</i> (Summerh.) P. J. Cribb & A. C. Podzorski (1)	Kew DNA 17863, Chase 17863 (K)	HQ222139	HQ222171	GQ145208
<i>Sirhookera lanceolata</i> (Wight) Kuntze	Kew DNA 15748, Chase 15748 (K)	HQ222152	HQ222258	GQ145211

Explanations: ^a – the sequence from Przemysław Baranow (Ph.D. thesis 2010, unpubl.); * – sequences from Russell *et al.* (2010)