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A Peculiar Amphitropical Genus of Paniceae (Poaceae, Panicoideae)

Systematic Botany

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A Peculiar Amphitropical Genus of Paniceae (Poaceae, Panicoideae)

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Abstract— The genus *Louisiella* (Poaceae, Paniceae) is reviewed. This African monotypic genus was originally circumscribed on the basis of morphological characters. Recent phylogenetic studies have shown its affinity with an American species of *Panicum*, *P. elephantipes*. Molecular and morphological, including anatomical, characters of *Louisiella fluitans* and *P. elephantipes* were here analyzed. Both species share vegetative and reproductive characters, including habit, ligules, blades, inflorescence, and spikelet features, such as a reduced lower glume, lanceolate upper glume and lower lemma, longer than the upper antheridium, lower palea reduced or absent and lower flower absent, upper antheridium indurate, and a caryopsis with a linear hilum; anatomically, both taxa are Kranz of the PS-subtype, with specialized chloroplasts on the outer parenchymatous sheath. The molecular phylogenetic analysis also corroborates

the relationship between these species, with 100% of branch support. As a result, we conclude that *P. elephantipes* has to be transferred to the herein amphitropical genus *Louisiella*. Affinities of *Louisiella* with other genera of Paniceae are also discussed. Finally, two lectotypes are designated for *Panicum fistulosum* and *Digitaria megapotamica*.

Keywords- *Louisiella*, Paniceae, *Panicum*, phylogeny, taxonomy.

During a revision of the genus *Panicum* L. at a worldwide level (Zuloaga in prep.), it became evident the morphological similarity of an American species of this genus, *P. elephantipes* Nees ex Trin., with a monotypic endemic genus from Africa, *Louisiella* C.E. Hubb. & J. Léonard. *Panicum elephantipes* is a common aquatic grass growing in ponds and shallow water (Hitchcock and Chase 1910; Zuloaga 1989), widely distributed from Mexico to Argentina. The species has been classified, within *Panicum*, in sect. *Dichotomiflora* (Hitchc.) Hitchc. & Chase ex Honda, sharing, with other species of *Dichotomiflora*, ligules membranous-ciliate, spikelets glabrous, first glume reduced, and upper anthercium smooth and shining (Hsu 1965; Zuloaga 1989). When describing the genus *Louisiella*, Hubbard and Léonard (1952) emphasized its aquatic habitat, the particular spikelet of the genus and the presence of a linear hilum. These authors mentioned that *Louisiella* was somehow related, in tropical Africa, with the genus *Acroceras* Stapf, although they stressed, in the latter genus, the laterally compressed crested tips of the upper anthercium, and presence of a lower palea and lower flower. Hubbard and Léonard (1952) also indicated that *Louisiella* was morphologically related to the American genus *Oplismenopsis*

Parodi, the latter being distinguished by these authors by its broader lanceolate to oblong-lanceolate leaf-blades, simple and longer panicle branches, subulate-acuminate awned spikelets, awned glumes, longer lower glume about half the length of the spikelet, short stout rachilla-internode between the upper glume and lower lemma, lower flower male with a well developed palea, and a lanceolate caryopsis. Clayton and Renvoize (1986) related the genus, in a diagram of relationships in Paniceae subtribe Setariinae, to *Oplismenopsis*, *Chaetium* Nees, and *Oryzidium* C.E. Hubb. & Schweick.. Recently, Morrone et al. (2012) treated *Louisiella* in tribe Paniceae, subtribe Panicinae, together with species of *Panicum* s. s. and *Yakirra* Lazarides & R.D. Webster; these authors mentioned that the genus is, based on molecular and morphological characters, within *Panicum* s. s. in a strongly supported clade with *P. elephantipes*.

In spite of the disjunct distribution, it is remarkable that both *Louisiella fluitans* and *P. elephantipes* share being aquatic herbs with spongy culms, sheaths with aerenchyma, ligules membranous-ciliate, spikelets lanceolate with the lower glume reduced, upper glume and lower lemma longer than the upper antheridium, lower palea reduced or absent, lower flower absent, and caryopsis with a linear hilum.

The purpose of this work is to confirm the phylogenetic relationships of these taxa, to discuss their affinities with *Panicum* s. s. and other genera of the Paniceae, and to classify them within the tribe. In order to accomplish this, we sampled additional material and analyzed them in conjunction with the data published by Morrone et al. (2012).

MATERIALS AND METHODS

Morphological Analyses— Morphological studies were based on herbarium specimens from CEN, COL, CTES, F, G, GH, IAN, LIL, LP, LPB, MEXU, MO, MY, NY, P, PORT, R, RB, SI, US, and VEN (Thiers 2013 [continuously updated]). For anatomical studies the second leaf below the inflorescence was selected. Specimens compared were *Fotius 2373*, *Fay 6162*, *Raynal 12822* of *L. fluitans*, and *Zuloaga 3083*, *Morrone 5191*, *Sanjines 253*, *Mac Dougal 3101* of *P. elephantipes*. Cross-sectional leaf anatomy was determined from hand-sectioned leaf blades of herbarium specimens previously was hydrated. The blades were sectioned approximately one-third from the base, after stained with safranin, and mounted in glycerin jelly. Epidermal preparations for light microscope studies were made following the methodology of Metcalfe (1960). The epidermis was stained in safranin. Anatomical descriptions were prepared using the terminology described in Ellis (1976, 1979).

Molecular Analyses— *TAXON SAMPLING*— Two new specimens, *P. elephantipes* (*Morrone 4486*) and *Louisiella fluitans* (*Raynal 12822*), were analyzed in this work. The two sequences obtained were included in a *ndhF* matrix containing a representative selection of Panicoideae taxa, extracted from the matrix of Morrone et al. (2012). The sequences of *Yakirra australiensis* from Morrone et al. (2012) was corrected, since some edition errors were detected in the published sequence while conducting this study; therefore, a new accession number was generated for this species (see Appendix 1) . The final matrix contained a total of 81 taxa. Detail of taxa analyzed and GenBank numbers are available in Appendix 1.

DNA SEQUENCING— Total genomic DNA was extracted from the silica-dried leaves with cetyl-trimethylammonium bromide protocol (Doyle and Doyle 1987). For herbarium specimens DNA was isolated using the DNeasy plant mini kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. The complete plastid gene of *ndhF* (ca.2,100 bp), was amplified by polymerase chain reaction (PCR) using primers specified by Olmstead and Sweere (1994) and Aliscioni et al. (2003). Four fragments were amplified (5F-536R, 536F-972R, 972F-1666R and 1666F-3R). The PCR reactions were performed in 25 μ L final volumes with 50-100 ng of template DNA, 0.2 μ M of each primer, 25 μ M dNTP, 5 mM MgCl₂ 1 \times buffer and 0.3 units of Taq polymerase from Invitrogen Life Technologies (Brazil). For most of the species PCR was carried out using the following parameters: 1 cycle of 94°C for 5 min, 39 cycles of 94°C for 30 s, 48°C for 1 min, and 72°C for 1 min 30 s, and a final extension cycle of 72°C for 10 min. PCR products were run out on a 1% TBE agarose gel stained with SYBR Safe DNA gel stain (Invitrogen) and visualized in a blue light transilluminator. Automated sequencing was performed by Macrogen, Inc. (Seoul, Korea). Forward and reverse strands were sequenced of each template to reach the complete sequence of each voucher. Alignment was manually performed, using BioEdit ver. 5.0.9 (Hall 1999).

PHYLOGENETIC ANALYSES— A maximum parsimony (MP) analysis was performed using TNT ver. 1.1 (Goloboff et al. 2008). All characters were equally weighted, treated as unordered, and gaps were scored as missing data. Prior to heuristic searches, all uninformative characters were deactivated. The searches involved 1,000 replicates, each of which generated a Wagner tree using a random addition sequence of taxa from the data matrix, swapping the initial tree

with TBR (tree bisection and reconnection) and retaining a maximum of 10 trees in each replicate. Subsequently, all optimal trees were swapped using TBR, holding a maximum of 20,000 trees. Branches with ambiguous length of 0 or 1 were collapsed, according to collapsing rules. A strict consensus tree was generated from the most parsimonious trees. Branch supports were estimated using the bootstrap (Felsenstein 1985) and a total of 10,000 replicates. Each replicate was analyzed using 10 Wagner trees as starting point followed by TBR branchswapping, saving only one tree per replicate. The *ndhF* matrix and the analysis performed can be found in TreeBase (study number 14698).

A Bayesian phylogenetic approach was also performed. Modeltest 3.7 (Posada and Crandall 1998), using Akaike information criterion, was used to determine the evolution model that best fit the data. Four Markov chains were run simultaneously in two independent runs for five million generations in MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001). Trees were sampled every 1,000 generations. Convergence diagnostics for log likelihood values were assessed visually using Tracer v.1.5.0 (Rambaut and Drummond 2007).

Posterior probabilities (PP) were determined after a burn-in of 25% of the trees of each run.

RESULTS AND DISCUSSION

Molecular Analyses— The *ndhF* complete data matrix consisted of 81 taxa×2,061 characters, with 338 informative positions. Percentage of missing data was 2.36%. Parsimony analysis resulted in 1458 most parsimonious trees,

998 steps long with consistency index (CI) = 0.45 and retention index (RI) = 0.76. The strict consensus tree is shown in Fig.1.

Bayesian approach generated a consensus tree shown in Fig. 2.

Parsimony and Bayesian consensus trees resulted fully congruent. Both trees showed *Louisiella fluitans* forming a clade with *Panicum elephantipes* with 100 % of bootstrap values (Fig.1) and PP = 1 (Fig. 2). Molecular characters shared by both taxa include four synapomorphies at 279, 540, 1,260 and 1,431 positions of the *ndhF* matrix.

The clade *L. fluitans* - *P. elephantipes* appeared in both analyses, with maximum branch support (bootstrap = 100% and PP =1), as the sister group of *Panicum s. str.*, within the subtribe Panicinae, of Paniceae.

The molecular phylogeny of Panicoideae of Morrone et al. (2012) considered within Panicinae, the genera *Louisiella*, *Panicum s. s.*, and *Yakirra*; with *Louisiella* included within *Panicum s. s.*, in a strongly supported clade with *P. elephantipes*, and *Yakirra* as the sister group of *Panicum s. s.* On the contrary, our analysis concludes, based on additional samples, that the pair *L. fluitans* - *P. elephantipes* is sister to the *Panicum s. s.* clade, which included the genus *Yakirra*. The placement of *Yakirra* within *Panicum s. s.* is in agreement with the results obtained in GPWG II (2012) with a different voucher.

As established in previous treatments (Zuloaga et al. 2006, 2007, 2010, 2011; Morrone et al. 2007, 2008; Sede et al. 2008, 2009), *Panicum* has to be restricted to its type subgenus, i.e., including only species with the following characteristics: annual or perennial cespitose plants with membranous-ciliate or ciliate ligules, open and lax inflorescences, ellipsoid to long-ellipsoid spikelets, with the upper antherium indurate, without multicellular microhairs, and with

simple or compound papillae at the top of the palea; also, all species of *Panicum* s. str. differ in their C₄ carbon fixation type, i.e. in having the NAD-me subtype (Zuloaga 1987), and share a basic chromosome number of $x = 9$.

Panicum elephantipes differs, from other species of *Panicum* s. s., by having a caryopsis with a linear hilum, the hilum reaching nearly one half the length of the caryopsis, and by its lanceolate spikelets, with the upper glume and lower lemma much longer than the upper antherium; also, Nuñez (1952) reported a particular, and unique, chromosome number for this species of $2n = 30$, pointing out that this number differs from the typical found in *Panicum* s. s. and that it probably represents a secondary basic number of $x = 15$. *Panicum elephantipes* shares with *Louisiella fluitans* several morphological characters, such as the aquatic habit, with culms spongy, prostrate, decumbent and rooting at the lower nodes, sheaths with aerenchyma, ligules membranous-ciliate, panicles open with spikelets lanceolate, lower glume scale-like, nerveless to 1-nerved, upper glume and lower lemma acuminate, subequal, lower flower absent, upper antherium shorter than the upper glume and lower lemma, and caryopsis with a linear hilum. Both taxa differ, between each other, by the presence of pilose nodes in *Louisiella fluitans* (vs nodes glabrous in *P. elephantipes*), blades 4-19 × 0.3-1.1 cm (vs 20-50 × 0.7-3 cm), panicles 4-11 cm long (vs. 25-50 cm long), spikelets 7-8.2 mm long (vs. 3.3-5.6 mm long), and upper antherium with long whitish macrohairs at the tip of the lemma (vs. upper antherium glabrous).

Leaf Blade Anatomy— *LEAF BLADE IN TRANSVERSE SECTION*— Outline: broadly V-shaped, with slightly involute margins; leaf thickness 75-100 μm, arms of the lamina symmetrical. Ribs and furrows. slightly rounded adaxial ribs,

abaxial ribs and furrows indistinguishable, rib associated with first and second order vascular bundles. Keel: developed on the adaxial side and slightly rounded on the abaxial side, associated with colorless parenchyma and with one first-order and two second-order vascular bundles. Vascular bundle arrangement: five or seven second-order vascular bundles between contiguous first-order vascular bundles; all vascular bundles situated in the center of the blade. Vascular bundle structure: First-order vascular bundles circular in outline. Second-order vascular bundles vertically elongated in outline, with xylem and phloem tissue distinguishable. Vascular bundle sheath: Kranz outer bundle sheath with specialized chloroplasts centrifugally arranged, parenchyma bundle sheath of the first-order vascular bundles entire or with adaxial interruption of sclerenchyma girders, consisting of nine to ten rounded and inflated cells with chloroplast to the outside; parenchyma bundle sheath of second-order vascular bundles entire, formed by five to six rounded and inflated cells with chloroplast to the outside. Sclerenchyma: small, adaxial and abaxial girders associated with the first-order and second-order vascular bundles; fibers lignified, small, rounded sclerenchyma caps located in leaf margins. Mesophyll: chlorenchyma not radiately arranged, two or three chlorenchyma cells between consecutive vascular bundles. Adaxial epidermal cells: bulliform cells fan-shaped, in restricted groups of five to six cells, epidermal cells small, regular in outline, papillae present, prickles present or absent Abaxial epidermal cells: bulliform cells absent, cuticle thickened; papillae present, prickles present or absent (Fig. 3, A, D-E).

ABAXIAL EPIDERMIS IN SURFACE VIEW— Zonation: costal and intercostal zones distinguishable; costal zone with three to six rows of long cells;

intercostal zone consisting of six to eight rows of long cells. Intercostal cells: elongated, more than three times longer than wide, with parallel side walls, vertical end walls, and undulating anticlinal walls. Stomata complex: triangular shaped, 18-20 μm long, 17-20 μm wide, two longitudinal rows of stomata per intercostal zone. Intercostal short cells: either solitary transversely elongated. Microhairs: absent. Papillae: present or absent. Prickle hairs: absent or present, small and with base shorter than the stomata; barb longer than the base. Macrohairs: absent. Costal silica bodies: dumbbell-shaped; intercostal silica bodies tall and narrow, transversely elongated (Fig. 3, C).

ADAXIAL EPIDERMIS IN SURFACE VIEW— Epidermal cells similar to the abaxial surface, except for the presence of a central band of rectangular cells, two to four cells wide, in the intercostal zones. Papillae present (Fig. 3 B).

The Kranz syndrome is a remarkable feature in which biochemical and physiological characteristics of C_4 photosynthesis are correlated with a distinctive anatomical structure (Ellis 1977; Hattersley 1987). Our analysis concluded that both *L. fluitans* and *P. elephantipes* are C_4 species of the PS (XyMS+) anatomical subtype (Brown 1977; Hattersley and Watson 1976): these species have parenchymatous and mestome sheaths around the vascular bundles, the outer parenchymatous sheath with specialized chloroplasts centrifugally arranged; also, there are two to three chlorenchymatic cells between contiguous vascular bundles (Fig. 3 A, D-E). Brown (1977) reported for *P. elephantipes* a value of $\delta^{13}\text{C}$ of -14.2. Both *Louisiella fluitans* and *Panicum elephantipes* display very similar anatomy with six round and inflated Kranz cells surrounding the second-order vascular bundles; Kranz chloroplasts usually are found close to the outer tangential cell wall. In both examined taxa the

abaxial epidermis shows papillae evenly distributed. It is noteworthy to mention that these anatomical features are also present in species of *Panicum* sect.

Dichotomiflora (Ellis 1988).

Regarding the disjunct distribution of taxa here analyzed, Bouchenak-Khelladi et al. (2010) mentioned that it is most likely that tribe Paniceae dispersed from Africa to Australia and/or North America from the early Miocene. Considering both the molecular and morphological characteristics of the species that were the focus of this paper, we are proposing the transference of *Panicum elephantipes* to the genus *Louisiella*; the key below will aid in identifying both taxa in this genus.

TAXONOMIC TREATMENT

LOUSIELLA C.E. Hubb. & J. Léonard, Bull. Jard. Bot. État Bruxelles 22 : 317.

1952.— TYPE: *Louisiella fluitans* C.E. Hubb. & J. Léonard

Aquatic perennials; culms prostrate, decumbent and rooting at the lower nodes, then ascendent, succulent; internodes spongy, hollow, greenish to pale, glabrous; nodes compressed, dark; sheaths open, striate, longer or shorter than the internodes, with aerenchyma, pale, glabrous; ligules membranous-ciliate; collar brown; blades oblong-lanceolate to linear-lanceolate. Inflorescence an open panicle, with first and second order branches diverging from the main axis, lower branches pseudoverticillate to opposite, the upper ones alternate; main axis triquetrous, wavy, pulvini glabrous; spikelets solitary or paired on third order branches; pedicels scabrous, triquetrous. Spikelets lanceolate, green to

pale brown, dorsiventrally compressed, dissarticulating below the lower glume, glabrous, upper glume and lower lemma subequal, membranous, longer than the upper anthercium, acuminate; lower glume reduced, hyaline; lower palea reduced or absent, lower flower absent; upper anthercium smooth and shining, indurate, acuminate, with sparse macrohairs toward the apex or glabrous; palea 2-nerved, lanceolate; lodicules 2, conduplicate. Caryopsis plano-convex; hilum linear, nearly 1/2 or as long as the caryopsis, embryo less than half the length of the caryopsis.

A genus with two species, distributed in tropical and subtropical rain forests of Africa and America.

KEY TO THE SPECIES

1. Spikelets 7-8.2 mm long; lower palea absent; upper anthercium with sparse macrohairs at the apex; hilum linear, 2/3 to 1/1 the length of the caryopsis; nodes pilose; blades 4-19 cm long; panicles 4-11 cm long

L. fluitans

1. Spikelets (3.3-)4.5-5.6 mm long; lower palea present; upper anthercium glabrous; hilum linear, nearly 1/2 the length of the caryopsis; blades 20-50 cm long; panicles 25-50 cm long

L. elephantipes

1. ***Louisiella elephantipes*** (Nees ex Trin.) Zuloaga, comb. nov. *Panicum elephantipes* Nees ex Trin., Gram. Panic.: 206. 1826. —TYPE: BRAZIL. Guaporé, *G.H. von Langsdorff s.n.* (lectotype here designated: LE-TRIN-

0692.01!; isolectotypes: B, BAA-1825 !, fragment ex B, US-974407!,
fragment ex LE).

Panicum elephantipes Nees, Fl. Bras. Enum. Pl. 2(1): 165. 1829, nom. illeg.
hom. —TYPE: BRAZIL. Pará: habitat in sylvis udis Archipelagi Paraensis,
K.F.P. von Martius s. n. (holotype: M!; isotypes: BAA!, fragment, US-80633!,
fragment ex M).

Panicum fistulosum Hochst. ex Steud., Syn. Pl. Glumac. 1: 71. 1853. —TYPE:
SURINAM. without locality, Feb-Apr 1844, *A. Kappler 1434* (lectotype here
designated, P-00740856!; isolectotypes: FI!, G-00099629!, GOET-006769!,
JE-00001173!, MO-123220!, P-00731505!, P-00740860!, S, US-80616!,
fragment ex S).

Panicum firmum F. Aresch., Pl. Itin. Eugeniae: 116. 1910, hom. illeg. nom., non
Kunth 1829. —TYPE: ECUADOR. Guayas: circa Guayaquil, *N.J. Anderson*
s.n. (not located).

Panicum sucosum Hitchc. & Chase, Contr. U.S. Natl. Herb. 17: 475, f. 30. 1915.
—TYPE: MEXICO. Jalisco: Orozco, near Guadalajara, 29 Nov 1910, *A.S.*
Hitchcock 7379 (holotype: US-693325!; isotypes: GH!, NY!, SI!).

Digitaria megapotamica Mez, Bot. Jahrb. Syst. 56, Beibl. 125: 7. 1921. —TYPE.
PARAGUAY. Central. Río Paraguay, pres de Asunción, Apr 1874, *B.*
Balansa 21 (lectotype: here designated, P-00740858!; isolectotypes: BAA-
00000843!, G-00030268!, G-00009734!, K, P-00740859!, P-00740857!).

Robust perennials, culms 1-1.5 m tall; internodes 8-20 cm long, 0.8-2 cm in
diameter; nodes glabrous; sheaths 8.3-15.5 cm long, the margins membranous,
one occasionally pubescent; ligules arcuate, 2-4 mm long, with short hairs
behind at the base of the blades, collar brown to purple; blades oblong-
lanceolate to lanceolate, 20-50 × 0.7-3 cm, flat, subcordate at the base,

sparsely pilose on the adaxial surface to glabrous, the margins scabrous. Inflorescence a lax and diffuse terminal panicle; panicle pyramidal, 25-50×4-18 cm, pedicels 2-8 mm long. Spikelets (3.3)-4.5-5.6×0.8-1.3 mm; lower glume 0.8-1.5 mm long, 1/6 to 1/4 the length of the spikelet, nerveless to 3-nerved, the apex obtuse to acute; upper glume 4-4.8 mm long, (5-)-7-9 nerved, the midnerve scabrous toward the apex; lower lemma 4-5 mm long, 7-9 nerved, the midnerve scabrous toward the apex; lower palea 2.5-3.5×0.5 mm, hyaline, 2-nerved or absent; lower flower absent; upper antheridium lanceolate, 2.9-4.6×0.8-1.2 mm, acuminate, pale to brown, glabrous; upper lemma 7-nerved, the margins inrolled over the palea; lodicules 2, ca. 0.3 mm long. Caryopsis fusiform, 2.2-2.3×0.8-0.9 mm, whitish; hilum linear, nearly ½ the length of the caryopsis, embryo less than half the length of the caryopsis. Figure 4.

Distribution and Habitat— Widely distributed in tropical America, from Mexico and the Caribbean to South America, where it is found in Venezuela, Colombia, Guiana, Ecuador, Peru, Bolivia, Brazil, Paraguay, Uruguay, and Argentina. It is frequent in margins of streams and rivers and ponds (Fig. 7).

Common Names— Common names for this species are "Camalote", "Canutillo", "Carrizo", "Pasto embalsado", "Pasto cañita", "Pasto de estero".

Chromosome Number— The chromosome number for this species is $2n = 30$ (Covas 1949, Nuñez 1952).

Representative specimens examined— ARGENTINA. Buenos Aires. Pdo. Berisso: Isla Santiago, *Cabrera* 1416 (F, LP), 2147 (F, NY, R, SI), 4430 (GH, LP). Chaco. Dpto. 1º de Mayo: Puerto Antequera, *Schinini et al.* 24319 (CTES, LIL, SI). Corrientes. Dpto. San Martín: Colonia Carlos Pellegrini, *Quarín & Schinini* 1006 (CTES, LIL, NY). Distrito Federal. Bajos de Palermo, *Boelcke* 4869 (SI). Entre Ríos. Dpto. Gualeguaychú: Brazo Largo, *Zuloaga et al.* 2338 (MO, SI). Formosa. Dpto. Formosa: 23 km al N de Formosa sobre ruta 11, *Krapovickas* 1009 (LIL, SI). Santa Fe. Dpto. San Jerónimo: Isla del Molino,

Lewis 650 (LIL, SI). Santiago del Estero. Dpto. Capital: La Banda, Río Dulce, *Yanes s.n.* (LIL 561331).

BELIZE. Stann Creek: Stann Creek river, *Gentle 7847* (F, US).

BOLIVIA. Beni: Espíritu, *Beck 5300* (K, LPB, SI), *5577* (K, LPB).

BRAZIL. Amapá: Oiapoque, beira do Rio Oiapoque entre Oiapoque e igarape Taparabou, *Black 49-8536* (P). Ceará: Serra do Baturite, S. Inacio de Azevedo, *Eugenio 271* (RB). Mato Grosso: Ilha Camargo, 20 km S da São João, *Allem & Vieira 2568, 2569, 2577* (CEN). Mato Grosso do Sul: Corumbá, Corixo Mutum, estrada para Carandazal, *Pott et al. 3094* (CEN). Pará: Boa Vista, Rio Tapajós, *Swallen 3148* (RB, US). Rio Grande do Sul: Porto Alegre, *Clayton 4464* (K, NY). Roraima: Rio Mucajai, Col. Fernando Costa, *Black & Magalhaes 51-12868* (IAN, P).

COLOMBIA. Atlántico: al sur de Barranquilla, Río Magdalena, *Mora 1457* (COL). Bolívar: Mun. Achi, Corregimiento La Raya, ciénaga La Raya, refugio "El Paraiso", *Callejas et al. 4333* (MO). Córdoba: Ciénaga de Ayapel, *Idrobo & Cleef 6562, 6595* (COL). Meta: Río Meta, isla del Arenal, *Cuatrecasas 3792* (F, COL).

COSTA RICA. Limón: Barrio de Colorado Sur, *Pohl & Lucas 13026* (F).

CUBA. Habana: Laguna Ariguanabo, *León 13312* (US). Oriente: Río Cauto, border of the river, *Ekman 16121* (NY). Pinar del Río: Cabañas, in wet places towards Sierra de las Animas, *Ekman 10504* (US).

DOMINICAN REPUBLIC. Santo Domingo: Llano costero, Santo Domingo, shore of Río Ozama, *Ekman 11149* (US).

ECUADOR. Guayas: cerca de Guayaquil, *Mille 1097* (F).

EL SALVADOR. San Miguel: Laguna del Jocotal, 20 km S of San Miguel, *Fassett 28810* (F, US).

GUATEMALA. Alta Verapaz: Near mouth of Río Polochic, *Goll 35A* (US).

GUIANA. Banks of Essequibo river, *Gleason 891* (GH, NY).

FRENCH GUIANA. Riviere de Kaw, *Hoock 109* (P).

HAITI. Swampy land near mouth of the Grand Rivière de Jérémie, southern peninsula, *Bartlett 17550* (US).

HONDURAS. Comayagua: Lago Yojoa, Pito Solo, emergent plants near shore, *MacDougal et al. 3057* (MO). Santa Bárbara: Agua Azul, N of Yopoa Lake, *Pohl & Gabel 13406* (F, MO).

JAMAICA. Middle Quarters, between Ipswich and Black River, *Hitchcock 9586* (MO, NY, P, US).

MEXICO. Distrito Federal: valley of Mexico, *Pringle 6322* (F, G, MEXU, MO, NY, P). Guanajuato: Lago de Yuriria, *CMVA 552* (MEXU). Hidalgo: Tula, *Matuda 25935* (MEXU). Jalisco: 5 miles SE of Guadalajara on road to Aguas Calientes, *Soderstrom 659* (US). Michoacán: Lago de Pátzcuaro, en el canal de San Pedrito, *Novelo 496* (MEXU).

PANAMA. Panama: Macapale Island in Madden Lake, *Tyson 5503* (MO).

PARAGUAY. Central: esteros a orillas del Río Salado, *Hassler 75* (SI).

PERU. Loreto: Río Nanay, Morona Cocha, *Rimachi 3603* (F, MO, NY).

PUERTO RICO. Vicinity of San Juan, water of swamp between Cataño and Bayamón, *Chase 6407* (US).

URUGUAY. Without locality, *Arechavaleta s.n.* (RB).

VENEZUELA. Amazonas: Depto. Río Negro, alrededores de las orillas del Bajo Casiquiare, entre Isla de la Paloma y Piedra, Guachapito, *Stergios et al. 8060* (PORT). Apure: Sabanas de los ejidos de Mantecal, *Ramia 4071* (MY, VEN). Bolívar: Ciudad Bolívar, *Bunting 3107* (MO). Delta Amacuro: Depto. Tucupita, along Brazo Macareo, near Boca del Macareo, S of Coporito and SE of Guara, *Steyermark et al. 115262* (MO, NY, VEN). Zulia: Caño Riccito, sur del Lago de Maracaibo, *Díaz 70* (MY).

According to Palacios (1969) this species is an excellent pasture in aquatic habitats.

2. *Louisiella fluitans* C.E. Hubb. & J. Léonard, Bull. Jard. Bot. État Bruxelles

22: 317. 1952. —TYPE: DEMOCRATIC REPUBLIC OF CONGO.

Yangambi, near Stanleyville, marais au centre de l'île en face de Bohonde, 17 July 1948, *J. Leonard 1798* (holotype: BR-000000758538!; isotypes: K-000282272!, K-000282273!, K-000282274!, YBI-165540134!).

Aquatic perennials; *culms* prostrate; internodes 2-10 cm long, 5-7 mm in diameter; nodes covered with whitish hairs up to 3 mm long; *sheaths* 1.5-4 cm long, the margins membranous, the upper ones ciliate; *ligules* 0.6-1 mm, membranous portion 0.3-0.6 mm long; collar sparsely pilose to glabrous; *blades* lanceolate to linear-lanceolate, 4-19×0.3-1.1 cm, flat, glabrous, rounded at the base, the apex acute, margins scabrous, the lower ones ciliate or not.

Inflorescence a panicle partially included or not in the upper leaves, 4-11×3-10 cm; pedicels 1-2.2 mm long. *Spikelets* 7-8.2×1-1.1 mm; *lower glume* 0.5-1 mm, 1/10-1/7 the length of the spikelet, nerveless to 1-nerved, obtuse; *upper glume* 7-8 mm long, acuminate, 7-9-nerved, the midnerve scabrous on the upper half; *lower lemma* glumiform, 7-9-nerved; *lower palea* and *lower flower* absent; *upper anthercium* narrowly ovoid, 4.5-5.2×1-1.1 mm, pale to brown, upper lemma 5-nerved, inrolled over the palea, with long whitish macrohairs towards the apex and bicellular microhairs on the ventral margins; palea with sparse macrohairs at the apex, otherwise glabrous; lodicules ca. 0.4 mm long. *Caryopsis* narrowly ellipsoid, 2.5-3×0.9-1.5 mm, whitish; hilum linear, 2/3 or as long as the caryopsis, embryo 1/3 the length of the caryopsis.

Distribution and Habitat— This species grows in aquatic habitats of Cameroon, Central African Republic, Southern Sudan, and the Democratic Republic of Congo. Hubbard and Léonard (1952) indicated that it is adapted to “the seasonal variations of the water level and to the raising of the substratum. With the rising of the water level, the stem-tips are raised by growth and especially by the straightening of the lower spreading portions. During the high water period, the plant is more or less entirely submerged and deposits of silt cover the lower portions rooted in the mud. As the level of the water falls, the upturned stems sink progressively lower and lower and spread out on the water surface, where they continue to grow, branch out in every direction and, at the nodes, put forth abundant hairy roots up to 40 cm long, while their lower portions, previously free-floating, spread out and take root on the recent mud layer” (Fig. 5).

Common Name— The common name for this species is “Koko”, according to the type specimen.

Representative specimens examined— CAMEROON. Quibi, 30 km SSW of Bongor, *G. Fotius* 2373 (P); Zoulla, 5 km NE of Yagoua, *Raynal* 12822 (P).

CENTRAL AFRICAN REPUBLIC. Parc Manovo Gounda, St. Floris, Gounda River, 1 km S of Gounda Camp, *Fay* 7353 (MO, P).

SOUTHERN SUDAN. Bahr El Ghazal, Lake Yiröl, *Myers* 13889 (K).

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Trichanthecium, a new genus segregated from *Panicum* (Poaceae, Paniceae) based on morphological and molecular data. *Systematic*

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APPENDIX 1. List of taxa used in the molecular analysis and GenBank accession numbers. New sequences are in bold, indicated with *, and voucher information is given.

Tribe Paniceae. *Acroceras zizanioides* (Kunth) Dandy, AY029618; *Cenchrus ciliaris* L., AY029625; *C. setaceus* (Forssk.) Morrone, AY029673; *Dichantherium acuminatum* (Sw.) Gould and Clark, AY188485; *Digitaria setigera* Roth ex Roem. and Schult., AY029629; *Echinochloa colona* (L.) Link, AY029631; *Eriochloa punctata* (L.) Desv., AY029634; *Louisiella fluitans* C.E. Hubb. & J. Léonard, JN604693; ***L. fluitans****, **Raynal 12822, KF938889**; *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs, AY029649; *Melinis repens* (Willd.) Zizka, AY029675; *Moorochloa eruciformis* (Sm.) Veldkamp., AY188452; *Oplismenus hirtellus* (L.) P. Beauv. AY029644; *Panicum*. Section *Dichotomiflora* (Hitchc.) Hitchc. & Chase ex Honda. *Panicum aquaticum* Poir., AY029658; *P. dichotomiflorum* Michx., AY188466; *P. elephantipes* Nees ex Trin., AY029647; ***P. elephantipes****, **Morrone 4486, KF938890**; *P. gouinii* E. Fourn., AY188467; *P. repens* L., AY029651. Section *Panicum*. *P. bergii* Arechav., AY188457; *P. fauriei* Hitchc., AY029650; *P. miliaceum* L., AY188472; *P. nephelophilum* Gaudich., AY029645; *P. stramineum* Hitchc. and Chase, AY188489. Section *Rudgeana* (Hitchc.) Zuloaga. *P. cervicatum* Chase, AY188459; *P. rudgei* Roem. and Schult., AY029661. Section *Urvilleana* (Hitchc.) Pilg. *P. chloroleucum* Griseb., AY188460; *P. racemosum* (P. Beauv.) Spreng., AY188481. Section *Virgata* Hitchc. and Chase ex Pilg. *P. tricholaenoides* Steud., AY188493; *P. virgatum* L., U21986. Ungrouped. *P. mystasipum* Zuloaga and Morrone, AY188474; *P. olyroides* Kunth, AY188475. *Panicum* "incertae sedis". Section *Clavelligera* Stapf. *P. adenophorum* K. Schum., AY188454; *P. claytonii*

Renvoize, AY188462. Section *Monticola* Stapf. *P. millegrana* Poir., AY029660. *P. trichanthum* Nees, AY188492. Section *Verrucosa* Hitchc. and Chase ex C. C. Hsu. *P. verrucosum* Muhl., AY188496. Ungrouped. *P. antidotale* Retz., AY188456; *Parodiophyllochloa cordovens* (E. Fourn.) Zuloaga and Morrone, AY188463; *Paspalidium geminatum* (Forssk.) Stapf, AY029662; *Pseudechinolaena polystachya* (Kunth) Stapf, AY029676; *Sacciolepis indica* (L.) Chase, AY029677; *Setaria lachnea* (Nees) Kunth, AY029683; *S. viridis* (L.) Beauv., U21976; *Stenotaphrum secundatum* (Walter) Kuntze, AY029684; *Trichantheium cyanescens* (Lam.) Zuloaga and Morrone, AY188465. *T. parvifolium* (Lam.) Zuloaga and Morrone, AY188476; *Urochloa plantaginea* (Link) R. D. Webster, AY029693; *Zuloagaea bulbosa* (Kunth) Bess, AY029648.

***Yakirra australiensis* * (Domin) Lazarides & R.D. Webster, Latz 12164, KF938888. Tribe Paspaleae. *Altoparadisium chapadense* Filg. et al., AY029619; *Anthaenantia lanata* (Kunth) Benth., AY029640; *Arthropogon villosus* Nees, AY029622; *Coleataenia prionitis* (Nees) Soreng, AY029652; *C. tenera* (Beyr. ex Trin.) Soreng, AY188491; *Cyphonanthus discrepans* (Döll) Zuloaga & Morrone, DQ646392; *Echinolaena inflexa* (Poir.) Chase, AY029633; *Homolepis glutinosa* (Sw.) Zuloaga and Soderstr., AY029637; *Hopia obtusa* (Kunth) Zuloaga and Morrone, AY029659; *Hymenachne donacifolia* (Raddi) Chase, AY029635; *H. pernambucensis* (Spreng.) Zuloaga, AY188478; *Ichnanthus pallens* (Sw.) Munro ex Benth., AY029638; *Mesosetum chaseae* Luces, AY029641; *Ocellochloa chapadensis* (Swallen) Zuloaga and Morrone, AY188486; *Oplimenopsis najada* (Hack. & Arechav.) Parodi, AY188453; *Otachyrium versicolor* (Döll) Henrard, AY029643; *Panicum incertae sedis*: Section *Tuerckheimiana* (Hitchc.) Zuloaga. *P. tuerckheimii* Hack., AY188494.**

Ungrouped. *P. hylaeicum* Mez, AY188470; *Paspalum conjugatum* Bergius, AY029669; *Phanopyrum gymnocarpon* (Elliott) Nash, AY188469; *Plagiantha tenella* Renvoize, AY029674; *Renvoizea sacciolepoides* (Renvoize & Zuloaga) Zuloaga & Morrone, EU107786; *R. trinii* (Kunth) Zuloaga and Morrone, EU107783; *Steinchisma hians* (Elliott) Nash, AY029685; *S. laxa* (Sw.) Zuloaga, AY029655; *Streptostachys asperifolia* Desv., AY029687. **Tribe Sacchareae.** *Cymbopogon flexuosus* (Nees ex Steud.) Will. Watson, AF117404; *Phacelurus digitatus* (Sibth. and Sm.) Griseb., AF117418, *Zea mays* L., U21985. **Tribe Thysanolaenae.** *Thysanolaena maxima* (Roxb.) Kuntze, U21984. **Tribe Zeugiteae.** *Zeugites pittieri* Hack., U21987.

FIG. 1. Parsimony strict consensus tree of *ndhF* sequences. Bootstrap supports are shown above branches. The position of *Louisiella* is indicated with a black bar.

FIG. 2. Bayesian tree of *ndhF* sequences. Posterior probabilities are shown above branches. The position of *Louisiella* is indicated with a black bar.

FIG. 3. Leaf blade anatomy and abaxial epidermis of *Louisiella elephantipes* and *Louisiella fluitans*. A-C. *Louisiella elephantipes*. A. Transverse section of blade showing first- and second-order vascular bundles. B. Adaxial epidermis. C. Abaxial epidermis. D-E. *Louisiella fluitans*. Transverse section of blade showing first and second-order vascular bundles (A, based on *Zuloaga 3083*, B-C, based on *Sanjines 253*, D, based on *Fay 6160*, E, based on *Raynal 12822*). Scales: A-E, bar: 50 μm .

FIG. 4. *Louisiella elephantipes*. A. Habit. B. Ligular region. C. Spikelet, lateral view. D. Spikelet, dorsal view. E. Spikelet, ventral view. F. Upper anthecium, ventral view. G. Lower palea. H. Caryopsis, embryo view. I. Caryopsis, hilum view (based on *Zuloaga et al. 2338*).

FIG. 5. *Louisiella fluitans*. A. Habit. B. Node. C. Ligular region. D. Portion of the inflorescence. E. Spikelet, dorsal view. F. Spikelet, ventral view. G. Upper lemma, ventral view. H. Upper palea. I. Caryopsis, embryo view. J. Caryopsis, hilum view (based on *Raynal 12822*).

FIG. 6. Map of Africa showing the distribution of *L. fluitans*.

FIG. 7. Map of America showing the distribution of *L. elephantipes*.