

# *Ancistrocladus ileboensis* (D.R.Congo), a new liana with unique alkaloids

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**Background and aims** – The taxonomy of *Ancistrocladus* Wall. (Ancistrocladaceae) is poorly understood. Recent interest is stimulated by the occurrence of biologically active naphthylisoquinoline alkaloids. The discovery of the here reported novelty was made in the framework of an interdisciplinary research program dealing with alkaloid patterns, chromosome numbers, demography, genetic variability, pollination biology, and phylogenetic relationships.

**Methods** – The morphological study was focussed on *A. ileboensis* and other related species occurring in the D.R.Congo.

**Key results** – The novelty *Ancistrocladus ileboensis* Heubl, Mudogo & G.Bringmann is described from the Congolian lowland forests ecoregion in the south of the Congo Basin. A distribution map and illustrations are provided. The new species is compared with four closely related taxa, from which it is distinct morphologically, chemically and biogeographically: *A. letestui* Pellegrin, *A. ealaensis* J.Léonard, *A. likoko* J.Léonard, and *A. congolensis* J.Léonard. Tables showing morphological and chemotaxonomic differences of the related taxa are presented.

**Conclusion** – The phytochemical peculiarities together with morphological features support the separation of *A. ileboensis* as a new species.

**Key words** – Ancistrocladaceae, *Ancistrocladus ileboensis*, D.R.Congo, Ilebo, naphthylisoquinoline alkaloids.

## INTRODUCTION

The taxonomy of the genus *Ancistrocladus* Wall. has to date been difficult to resolve and the delimitation of species is somewhat problematic, not only because it is an under-collected genus, but also because taxa are fairly rare and not flowering abundantly. Consequently the genus has for a long time received limited attention but with the discovery of numerous naphthylisoquinoline alkaloids which have shown significant antiparasitic activities and against HIV (Hallock et al. 1997), the genus became quite popular recently.

The Central African species of *Ancistrocladus* have been treated by Léonard (1949, 1982, 1986) and revised by Hutchinson & Dalziel (1927) and by Keay (1954) for the Flora of West tropical Africa. Besides these regional studies on the genus a synoptic revision of the African species was compiled for the first time by Cheek (2000). Recently Taylor et al. (2005) have presented a revision of the whole genus

based on an extensive examination of herbarium collections, giving detailed descriptions of taxa, information on type specimens and synonyms as well as on distribution, phenology, habit, and conservation status. Based on this updated account of the genus *Ancistrocladus*, sixteen species are recognized. Recently a new species, *Ancistrocladus benomensis* Rischer & G.Bringmann from Malaysia (Rischer et al. 2005), has been described.

All taxa of this rather small genus are scandent shrubs or woody lianas, climbing with tendril-like, sympodial shoots that bear recurved, circinate woody hooks, representing true sympodial stem apices. Other important diagnostic features are the minute epidermal pits densely distributed on both leaf surfaces as well as larger glands with a raised ring structure, which produce a mucilaginous, waxy secrete. Further peculiarities are the indehiscent nuts with persistent disk and style base, and the five accrescent sepals (papery fruit wings

that enlarge remarkably during development) and the deeply ruminant endosperm of the seeds.

The molecular and chemical investigations in this genus are part of an ongoing research project for a better understanding of phylogenetic relationships within the genus. It is an important task in our project to gain more basic biological data on naphthylisoquinoline patterns, chromosome numbers, demography, genetic variability, pollination biology, and phylogenetic relationships of this poorly known genus. The outlined interdisciplinary research program involves scientists from Germany (Systematic Botany / Molecular Phylogeny and Natural Products Chemistry) and the D.R.Congo, notably from the University of Kinshasa.

The present paper discusses and describes a novelty, *Ancistrocladus ileboensis*, discovered during field work for the program in the D.R.Congo.

## MATERIAL AND METHODS

The holotypes from BR have been checked only via digital pictures whereas the material from P was inspected during a

visit. Individuals were cultivated at the Botanical Garden of the University Würzburg.

## RESULTS AND DISCUSSION

### Phytogeography

The genus *Ancistrocladus* shows interesting distribution patterns with two centres of diversity in West and Central Africa (eleven species) and in SE Asia (six species). The majority of species in Africa occur in the evergreen forests of the Guineo-Congolian Region with two outliers, *A. tanzanien-sis* Cheek & Frim.-Møll. and *A. robertsoniorum* J.Léonard, which occur in East Africa (Tanzania and Kenya) (Léonard 1984, Freson 1967, Cheek et al. 2000). According to the distribution map provided by Freson (1967) and the phytochoria defined by White (1979) *Ancistrocladus* species avoid both the Dahomey and the Sangha River interval and are nearly absent from ecoregion 15 (Burgess et al. 2004). Concerning the locality of the novelty *A. ileboensis*, there is a gap to the other Congolian species and it is clearly separated from *A. letestui* (fig. 4).

**Table 1 – Comparison of diagnostic characters of the species distributed in the D.R.Congo.**

Based on own data and information according to Taylor et al. (2005).

|                                        | <i>A. ileboensis</i>                  | <i>A. congolensis</i>                     | <i>A. likoko</i>                                         | <i>A. ealaensis</i>                                   |
|----------------------------------------|---------------------------------------|-------------------------------------------|----------------------------------------------------------|-------------------------------------------------------|
| <b>Leaves</b><br>(at branchlet apices) |                                       |                                           |                                                          |                                                       |
| size                                   | 9–25 × 2.5–5 cm                       | 7–27 × 3–10 cm                            | 8–40 × 3–13 cm                                           | (3–)5–25 × 2.2–8.1 cm                                 |
| apex                                   | obtuse to rounded, no distinct acumen | obtuse or abruptly acuminate, acumen 5 mm | obtuse to rounded, shortly acuminate, acumen 1–2(–10) mm | usually acute to long-acuminate, acumen 10–25(–40) mm |
| secondary veins                        | 4–20(–30) pairs                       | (4–)10–14 pairs                           | 9–15 pairs                                               | 5–12 pairs                                            |
| branchlet leaves L/W                   | 3.7–6.2                               | 2.0–3.4                                   | 2.1–3.5                                                  | 2.1–4.6                                               |
| size larger pits (diameter)            | 0.8–1.1(–1.4) mm                      | 0.2–0.4 mm                                | 0.3–0.4 mm                                               | 0.3–0.5 mm                                            |
| <b>Inflorescence</b>                   | 6–10 × 6–13 cm                        | 4–5.5 × 5.5–12 cm                         | 6–24 × 4–5 cm                                            | 3–6 × 4–7 cm                                          |
| <b>branchlets</b>                      | with hooks                            | without hooks                             | with hooks                                               | without hooks                                         |
| <b>Flower diameter</b>                 | 6.8–8.0 mm                            | 8–10 mm                                   | 12–20 mm                                                 | 8–12 mm                                               |
| <b>Petal colour</b>                    | bright red                            | yellow                                    | cream-yellow                                             | golden-yellow                                         |
| <b>Sepals</b>                          | 2.4–3.9 × 1.9–2.4 mm                  | 2.5–6 × 2–3 mm                            | 5–9 × 3–6 mm                                             | 2.5–5 × 2–3 mm                                        |
| <b>Petals</b>                          | 2.2–3.0 × 2.1–3.0 mm                  | 4 × 3.0–3.5 mm                            | 3–4 × 3–4 mm                                             | 2–3 × 2–3 mm                                          |
| <b>Stamens</b>                         | 2 whorls, unequal                     | 1 whorl                                   | 2 whorls, unequal                                        | 1 whorl, unequal                                      |
| outer                                  | 1.4–1.7 mm                            | all equal length                          | 1.2 mm                                                   | 0.8–1.0 mm                                            |
| inner                                  | 0.9–1.2 mm                            | 2.0 mm                                    | 0.4 mm                                                   | 0.5–0.7 mm                                            |
| <b>Styles</b>                          |                                       |                                           |                                                          |                                                       |
| number                                 | 3                                     | 3                                         | 1                                                        | 1                                                     |
| length                                 | 1.2–1.6 mm                            | c. 1.5 mm                                 | 2–3 mm                                                   | 1–1.5 mm                                              |
| <b>Fruits</b>                          |                                       |                                           |                                                          |                                                       |
| size                                   | 35–45 × 40–50 mm                      | 8–15 × 10–20 mm                           | 10–18 × 12–18 mm                                         | 4–5 × 7–8 mm                                          |
| ridges                                 | 5                                     | 5–7                                       | 10                                                       | 5                                                     |
| calyx lobes                            | unequal, 18–32 × 6–11 mm              | unequal, 5–10 × 3–4 mm                    | equal, 8–12 × 4–7 mm                                     | unequal, 5–10 × 4–8 mm                                |
| <b>Seeds (diameter)</b>                | 3–4 mm                                | 6–7 mm                                    | 7 × 9–10                                                 | c. 6 mm                                               |

**Table 2 – Comparison of the naphthylisoquinoline alkaloid pattern of the Congolese *Ancistrocladus* species.**

Data from Bringmann et al. 1995, 2000a, 2000, 2002, 2003a, 2003b, Unger et al. 2004.

<sup>a</sup> For *A. ealaensis* and *A. congolensis*, several most peculiar, unprecedented structures were described in the literature in the early 70s, i.a. without a methyl group at C-1, without an oxygen function at C-5', and/or with an – expectedly highly reactive – 1,2-dihydroisoquinoline portion. Because of the lacking spectroscopic evidence, the proposed structures, e.g. for ancistrocogine (Foucher et al. 1975), and due to the fact that the results of these early investigations could not be reproduced at all in recent phytochemical analyses of these lianas, these speculative structures were not included into this chemotaxonomic examination.

<sup>b</sup> n.d. = not yet determined.

|                                     | <i>A. ileboensis</i>                                                                                      | <i>A. congolensis</i> <sup>a</sup>                 | <i>A. likoko</i>                                                                 | <i>A. ealaensis</i> <sup>a</sup> |
|-------------------------------------|-----------------------------------------------------------------------------------------------------------|----------------------------------------------------|----------------------------------------------------------------------------------|----------------------------------|
| <b>Position of axis</b>             | 3 × 5,1'<br>3 × 7,1'                                                                                      | 4 × 5,8'<br>1 × 7,1'<br>1 × N,C                    | 6 × 5,8'                                                                         | 2 × 5,8'<br>1 × N,C              |
| <b>Axial configuration</b>          | 3 × P<br>1 × M<br>2 × n.d. <sup>b</sup>                                                                   | 4 × P<br>1 × M                                     | 4 × P<br>2 × M                                                                   | 2 × P                            |
| <b>Type of alkaloid</b>             | 1 × Ancistrocladaceae-Dioncophyllaceae hybrid type<br>3 × Ancistrocladaceae type<br>2 × n.d. <sup>b</sup> | 5 × Ancistrocladaceae-Dioncophyllaceae hybrid type | 4 × Ancistrocladaceae-Dioncophyllaceae hybrid type<br>2 × Ancistrocladaceae type | 2 × Ancistrocladaceae type       |
| <b>Degree of hydrogenation</b>      | 5 × tetrahydro<br>1 × dihydro                                                                             | 5 × tetrahydro                                     | 4 × tetrahydro<br>2 × dihydro                                                    | 1 × tetrahydro<br>1 × dihydro    |
| <b>N-Substituent</b>                | 5 × NH                                                                                                    | 3 × N-Me<br>2 × NH                                 | 2 × N-Me<br>4 × NH                                                               | 2 × NH                           |
| <b>Configuration at C-1 and C-3</b> | 2 × 1S, 3S<br>1 × 1R, 3S<br>1 × 3R<br>2 × n.d.                                                            | 5 × 1R, 3R                                         | 3 × 1R, 3R<br>1 × 1S, 3R<br>2 × 3S                                               | 1 × 1S, 3S<br>1 × 3S             |
| <b>Substituent at C-6</b>           | 6 × OMe                                                                                                   | 4 × OH<br>1 × OMe                                  | 4 × OH<br>2 × OMe                                                                | 2 × OMe                          |
| <b>Substituent at C-8</b>           | 6 × OMe                                                                                                   | 3 × OH<br>2 × OMe                                  | 2 × OH<br>4 × OMe                                                                | 2 × OMe                          |
| <b>Substituent at C-4'</b>          | 6 × OMe                                                                                                   | 5 × OMe                                            | 3 × OH<br>3 × OMe                                                                | 2 × OMe                          |
| <b>Substituent at C-5'</b>          | 6 × OMe                                                                                                   | 2 × OH<br>3 × OMe                                  | 2 × OH<br>4 × OMe                                                                | 1 × OH<br>1 × OMe                |
| <b>Further related compounds</b>    |                                                                                                           |                                                    | 1 naphthoquinone,<br>1 isoquinoline                                              | 3 naphthalenes                   |

### Diagnostic characters

No large-scale inventory of lianas has been so far conducted in the Congo Basin rain forest. Thus reliable data and informations are lacking and the role of lianas in this ecosystem, distribution patterns and diversity remains unclear. Consequently by increased collecting in this area more taxa as demonstrated for the genus *Ancistrocladus* will come to light.

When *A. ileboensis* was collected in the field, the sterile specimens were at first assigned to *A. letestui*, which is widespread from Nigeria and Gabon to the Republic of Congo (Brazzaville) and it was considered that this would be a new record for the D.R.Congo. Several new naphthylisoquinoline alkaloids had been identified (Unger et al. 2004), before it was realized that actually a new species was involved. Based on the successful cultivation of some individuals at the Botanical Garden of the University Würzburg, which developed flowers and fruits, we were able to confirm the status of this

new taxon. Samples of *A. ileboensis* were compared with isotype and holotype specimens of *A. ealaensis*, *A. congolensis*, and *A. likoko* deposited in the herbarium of the National Botanic Garden of Belgium (BR) and the Muséum National d'Histoire Naturelle Paris (P). These species had been described for the D.R.Congo by Léonard (1949) en bloc together with additional information on ecological and developmental observations. His regional study prepared for the Flore d'Afrique Centrale (Léonard 1982) is the most informative on *Ancistrocladus* concerning Central Africa. Though sharing many similarities with the geographically close *A. congolensis*, to which it is probably most closely related, *A. ileboensis* appears to be quite distinct from this species and from the other taxa from the D.R.Congo so far known: *A. ealaensis* and *A. likoko*. However, *A. letestui*, although widely distributed in West-Central Africa, is not recorded from the D.R.Congo.

According to the revision by Taylor et al. (2005) and the detailed descriptions provided for the species from Central

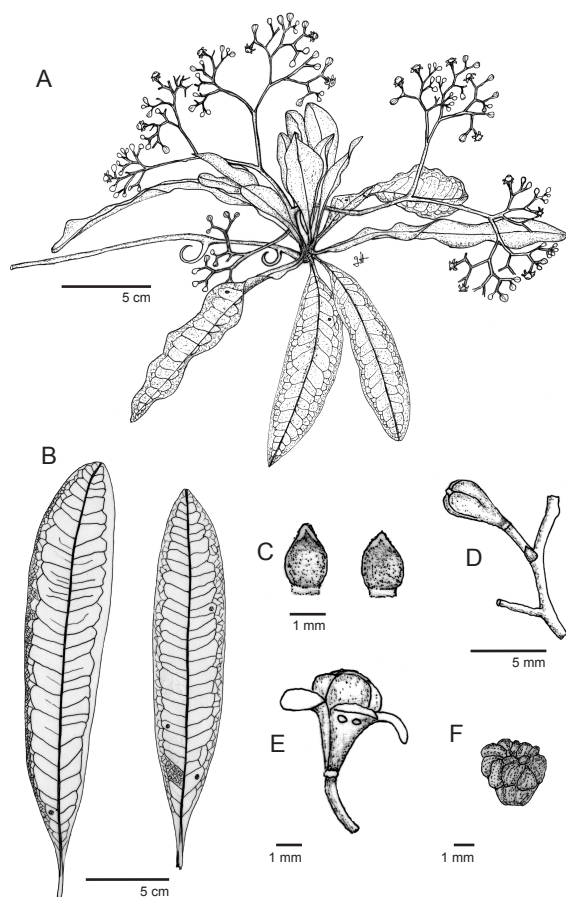


**Figure 1** – *Ancistrocladus ileboensis*: A, lateral branch with crowded leaves and inflorescence; B, leaves from main stem, venation in detail; C, bracts; D, flower in bud with bract; E, open flower with reflexed sepals; F, seed. Drawings by G. Heubl.

Africa our new species *A. ileboensis* can be distinguished from *A. letestui* by the following features (data for *A. ileboensis* in brackets): adult leaves with 1–3 large pits usually oblong (0.5–)2–4 × 0.5–1.0 mm in diameter (*A. ileboensis* 2–5 small pits, 0.8–1.1(–1.4) mm in diameter); branchlet leaves narrowly oblanceolate to elliptic-oblanceolate, ratio L/W 3.5–15.5 (*A. ileboensis* L/W 3.7–6.2); inflorescence bracts 3–3.5 mm, spatulate, elongated at the base, by late anthesis becoming cochleate to clavate, persisting through the fruiting stage (*A. ileboensis* bracts ovate 1.2–1.6 mm long, not persisting at fruit); petals cream (*A. ileboensis* red); bark of the stems often with small epidermal protuberances (*A. ileboensis* stem longitudinally fissured), fruiting sepals 21 × 8–10 mm (*A. ileboensis* fruiting sepals 35–45 × 40–50 mm).

From *A. korupensis* (distribution Nigeria and Cameroon) the new species can be delimited not only chemically but also concerning size of larger pits on adult leaves 0.4–0.6 mm (*A. ileboensis* 0.8–1.1 mm), sepals 5.0–6.0 × 3.0–4.0 mm (*A. ileboensis* 2.4–3.9 × 1.9–2.4 mm, not ciliate at apex), pedicels 6–9 mm (*A. ileboensis* 3–4 mm), larger sepals of

fruit 34–48 × 13–21 mm (*A. ileboensis* 30–35 × 9–11 mm), seeds c. 8 mm (*A. ileboensis* 3–4 mm).

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Only one single secondary metabolite has so far been described from *A. letestui*, viz. dioncophylline A, i.e. a Dioncophyllaceae-type naphthylisoquinoline alkaloid (Hallock et al. 1995)– while the alkaloid pattern of *A. ileboensis* was shown to consist of several naphthylisoquinolines, but dioncophylline A was not among them (Unger et al. 2004).

Morphologically, *A. ileboensis* is quite similar to *A. congolensis*, which is also characterised by the presence of three styles (instead of one in *A. likoko* and *A. ealaensis*). Table 1 serves to distinguish *A. ileboensis* from the other species in the D.R.Congo.

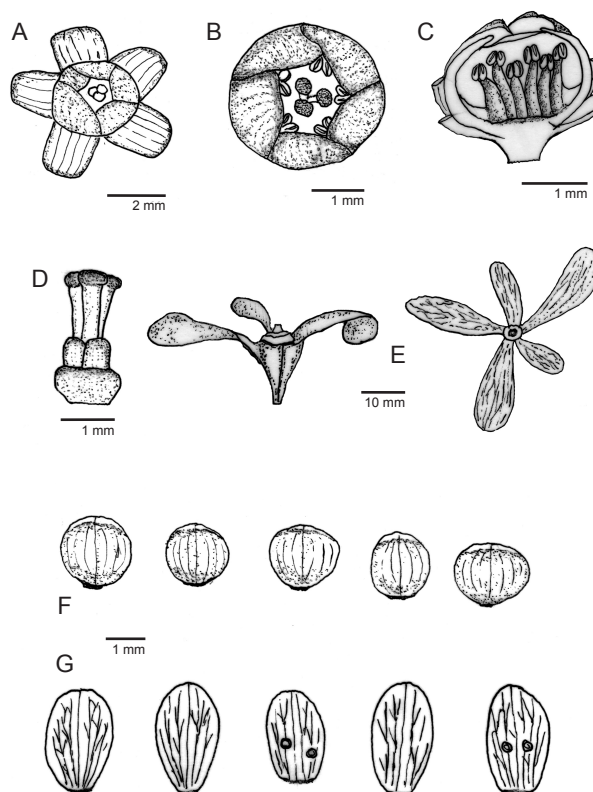


**Figure 2** – *Ancistrocladus ileboensis*: A, flower with reflexed sepals; B, view of petals, anthers and stigmas; C, flower longitudinally cut, part of perianth and adnate stamens, sepals removed; D, ovary with three styles; E, mature fruit with enlarged calyx wings; F, petals; G, sepals with glands. Drawings by G. Heubl.

### Chemotaxonomic demarcation

In recent years great interest in *Ancistrocladus* has arisen due to the discovery of many new naphthylisoquinoline alkaloids (Bringmann & Pokorny 1995, Hallock et al. 1995, Bringmann et al. 2000a, 2000b, Unger et al. 2004, Bringmann et al. 2004, Bringmann et al. 2006, Bringmann et al. 2008), which show a remarkable variation in structure and are, simultaneously, the very first acetogenic (i.e. acetate-derived) isoquinoline alkaloids (Bringmann et al. 2000a). Pharmacologically remarkable, some purified alkaloids provide promising antiparasitic activity against several pathogens of tropical infectious diseases (François et al. 1997, Bringmann et al. 2003a, Ponte-Sucre et al. 2007) and against HIV (Hallock et al. 1997).

It is of chemotaxonomical importance that only *Ancistrocladaceae* and *Dioncophyllaceae* are characterised by the possession of these remarkable metabolites, which confirms the close relationship of the two families as inferred from molecular analysis (Meimberg et al. 2000).

The metabolite pattern of *A. ileboensis* differs completely from those of the three other species distributed in the D.R.Congo. While *A. congolensis*, *A. likoko*, and *A. ealaensis* predominantly produce naphthylisoquinolines with the biaryl axis in 5,8'-position, no such 5,8'-coupled alkaloid has so far been found in *A. ileboensis*. In this new species, rather 5,1'- and 7,1'-coupled naphthylisoquinoline alkaloids have been detected (Unger et al. 2004). Table 2 highlights the differences concerning alkaloid patterns.

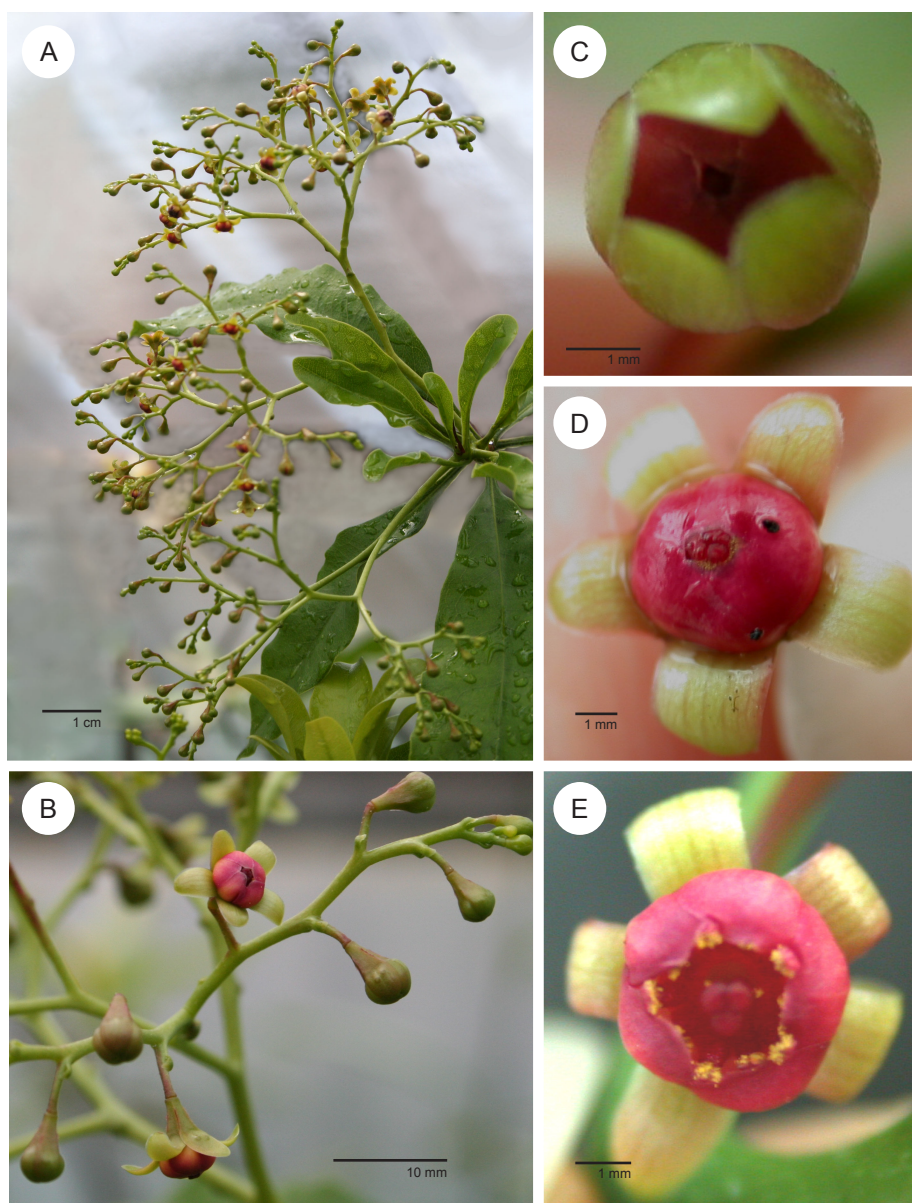


**Figure 3** – *Ancistrocladus ileboensis*: A, lateral branch with crowded leaves and inflorescence; B, cyme with flowers at beginning of anthesis; C, flower bud; D, open flower with reflexed sepals; E, flower at anthesis, fully developed. Photos by G. Heubl.

## TAXONOMY

### *Ancistrocladus ileboensis* Heubl, Mudogo & G. Bringmann, sp. nov.

Species nova habitu affinis *Ancistrocladus congolensis*, sed differt ramis lateralibus cum uncinis circinatis, foliis rosulatis ramorum lateralium angustioribus 2.5–5 cm, venis secundariis paribus 14–20(–30) dispositis, floribus minoribus, petalis rubris 2.2–3.0 × 2.1–3.0 mm, staminibus 10 in verticillis duos dispositis, inaequalibus, externis 1.4–1.7 mm longis, internis 0.9–1.2 mm, fructibus cum sepalis tenuibus, inaequalibus, majoribus spathulatis 32 × 11 mm, minoribus oblongis spathulatis 18 × 6 mm, seminis diametro 3–4 mm. A speciebus *Ancistrocladus ealaensis* atque *A. likoko* numero stylo- rum (1 non 3) discrepat. – Type: D.R. Congo, Ilebo region, near Bambange, 16 Mar. 2000, *Virima Mudogo* 57 (holo-: M; iso-: M).

**Liana** climbing in trees, bark of adult stems purplish-grey, smooth to longitudinally fissured, lateral branchlets each bearing 1–2 circinate hooks. Older saplings **leaves** 17–35 × 2.5–5.5 cm crowded at the end of the shoot, sessile, elliptic-obovate, at apex obtuse to subacute, at base narrowly cuneate, pseudopetiolate, midrib prominent beneath, secondary veins 14–20(–30) pairs, veins finely reticulate, prominulous on both surfaces, about 2–10 oblong or circular glands distributed 3–10 mm from the margin and along the midrib on the lower surface, small pits rather densely distributed on both surfaces, large pits 0.8–1.1(–1.4) mm diameter, circular, occasional and scattered on both surfaces (fig. 1B). Leaves drying discoloured, dark green-brown above, somewhat lighter brown beneath, small pits scattered on both surfaces. Leaves at branchlet apices near inflorescences 9–25 × 2.5–5.0 cm, sessile, elliptic-oblong, apex obtuse to acuminate, otherwise as described for the saplings leaves. **Inflorescence** paniculate, lateral or subterminal among leaves at apices of branchlets, these bearing 2–3 hooks, without bractal leaves. Cyme emerging from a leaf rosette, lax, branched portion corymbiform, 6–10 × 6–13 cm, 3–4 times dichotomously branched, bearing 30–60 flowers. Peduncle 2–4 cm long (part to first branching point). Pedicels 3–4 mm long, weakly articulated near the base. Bracts small, ovate, 1.2–1.6 mm long, at apex acute. **Flowers** all pedicellate, 5-merous, small, 6.8–8.5 mm in diameter at anthesis. Sepals 5, pale green, slightly

unequal, reflexed at anthesis, oblong to spatulate, straight at base, rounded at apex, shortly decurrent on ovary, with fine-reticulate venation, 2.4–3.9 × 1.9–2.4 mm, fleshy, glabrous, cuneate, two sepals of each flower abaxially with 0–2 cup-shaped to crateriform, often paired glands, calyx lobes somewhat longer as corolla, persistent and much enlarged in fruit. Petals 5, free, bright red, glabrous, convolute, 2.2–3.0 × 2.1–3.0 mm, suborbicular, convex, thickened towards base. Stamens 10, apparently in two whorls, alternately unequal, longer stamens (inner whorl) 1.4–1.7 mm long, shorter stamens 0.9–1.2 mm long, filaments fleshy, broadened at whole length, anthers 0.4–0.5 mm with thecae clearly separated by the connective. Ovary mostly semi-inferior, upper part superior, conical, styles 3, stout, red, 1.2–1.6 mm long, stigma circular. **Fruit** turbinate, nut, brown, persistent sepals spreading, papery-coriaceous, upper surface glossy, unequal, veins clearly prominent beneath, only major ones visible above, the 2 shorter slightly obovate to spatulate 18–22 × 6–8 mm, the 3 longer oblong to spatulate, 30–35 × 9–11 mm, bases of all wings adnate to the fruit, margins decurrent along the fruit body, longitudinally shallowly 5-ridged, the remnants of the style forming a short column above the spreading wings. **Seed** obconic, 3–4 mm diameter. Figs 1–3.

**Vernacular name** – Nhamu-ndop

**Distribution** – So far the species is only known from the type location. Fig. 4.

**Ecology** – The species occurs mainly in dry land forest at altitudes of 420 to 465 m, also in secondary forests, with clay soils.

**Etymology** – The species name refers to the type locality Bambange north of the town Ilebo (Province Kasai-Occidental) in the South-Central D.R. Congo. The town lies along the Kasai River near its junction with the Sankuru River.

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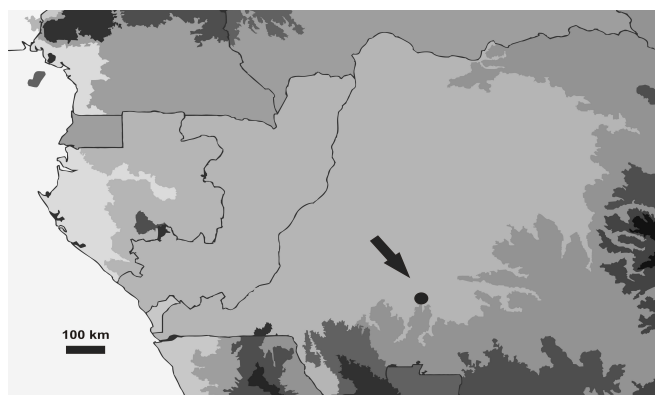


**Figure 4** – Type locality of *Ancistrocladus ileboensis* in the Democratic Republic of Congo.

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