*Phytotaxa* 204 (4): 265–276
www.mapress.com/phytotaxa/


**Article**

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.204.4.3

# Evolution of species diversity in the genus *Chamaecostus* (Costaceae): molecular phylogenetics and morphometric approaches

THIAGO ANDRÉ*[1,2], CHELSEA SPECHT[3], SHAYLA SALZMAN[4], CLARISSE PALMA-SILVA[5] & TÂNIA WENDT[2]
[*] *thiagojcandre@gmail.com; corresponding author*
[1] *Universidade Federal do Oeste do Pará, Herbário de Santarém (HSTM), Santarém (PA), Brazil*
[2] *Universidade Federal do Rio de Janeiro, Rio de Janeiro (RJ), Brazil.*
[3] *University of California, Berkeley (CA), USA.*
[4] *Harvard University, Cambridge (MA), USA.*
[5] *Universidade Estadual Paulista, Rio Claro (SP), Brazil*

## Abstract

While most species within the genus *Chamaecostus* (Costaceae) are well defined, the broad geographic range and long list of synonyms associated with *Chamaecostus subsessilis* led us to believe there may be some cryptic species within the complex. We thus investigate the phylogenetic relationships of species in the *Chamaecostus* lineage and specifically test the monophyly and diversity of the *Chamaecostus subsessilis* species complex from a population perspective by analyzing molecular sequence data and leaf morphometrics. We interpret evolutionary trends across the entire genus based on a molecular character-based phylogenetic hypothesis that includes all currently described species of *Chamaecostus*. Our results show that while *Chamaecostus* is strongly monophyletic, *C. cuspidatus* is found to be sister to a clade of some but not all samples of *C. subsessilis*, making it necessary to acknowledge more than one species in the *C. subsessilis* complex. Herbarium specimens of the *C. subsessilis* complex could be assigned based on geographic proximity to one of the major three clades recovered in the phylogenetic analysis. Leaf morphometric measurements were performed on each of these lineages and traits were tested to detect differences among phylogenetic lineages. We conclude by proposing the recognition of a new combination, *Chamaecostus acaulis*, which we describe.

**Keywords**: species complex, morphometrics, tropical plant taxonomy, spiral gingers, South America

## Introduction

Most species are identified on the basis of incongruent patterns and discontinuity of trait variation across individual specimens. Individuals comprising a species manifest their variability in rather continuous variation, and their integrity can only be understood by sampling through the range of this variation (Frost & Kluge 1994), which can then provide the basis for ascertaining trends across the species unit. These observed trends frequently support the recognition of taxa. However, morphological discontinuity does not always co-occur with lineage splitting: isolated subpopulations bearing significant genetic structure can lack phenotypic differences between them, making species delimitations challenging (DeSalle *et al*. 2005, Padial & De La Riva 2009, Padial *et al*. 2010, Florio *et al*. 2012). Such cryptic speciation is characterized by two or more morphologically indistinguishable groups of organisms that are found to belong to distinct evolutionary lineages (Sáez & Lozano 2005). Perceived cryptic speciation can also derive from our inability to distinguish important, and not always prominent, morphological differences (Shaffer & Thomson 2007). Using a phylogenetic lineage approach, species-level phylogenies and networks are able to provide a consistent and predictive evolutionary understanding of species limits by helping to identify unique evolutionary entities among population-level sampling (Funk & Omland 2003, Goldstein & DeSalle 2000). The phylogenetic approach to species delimitation is particularly promising because distinct species are interpreted as being on separate evolutionary trajectories (Hey & Pinho 2012), which, in some cases, are expected to continue to diverge even in the absence of reproductive barriers (Rieseberg *et al.* 2004).

Species of *Chamaecostus* Specht & Stevenson (2006: 157) are fairly distinguishable from one another, with the sole exception being the *C. subsessilis* (Nees & Mart.) Specht & Stevenson (2006: 158) species complex. Taxonomic

*Accepted by Maarten Christenhusz: 6 Mar. 2015; published: 8 Apr. 2015* 

disputes regarding *C. subsessilis* are largely due to its widespread distribution and the various subtle morphological differences that occur across its geographic range. Individuals placed within this group comprise the largest geographic range for any species of *Chamaecostus*, ultimately forming a species complex that encompasses eleven historically described species. Maas (1972, 1977) emphasized in his monographs that these eleven described species could not be separated from one another based on floral characters alone, which are rather constant across the geographic distribution. Previous taxonomic descriptions referred mainly to variation in vegetative characters such as plant height, leaf shape, and leaf hairiness. These characters were hypothesized by Maas (1972) to be driven by environmental factors specific to particular habitats, as he found no clear geographic isolation separating any of the described forms. Maas combined all eleven taxa under one species name (Maas 1972, 1976, 1977); initially Maas (1972) used *Costus warmingii* Petersen as described in Martius (1890: 57), but later (Maas 1976) revised the name based on the previously described type of *Globba subsessilis*, described by Nees and Martius (1823: 29) but not cited in Martius' Flora Brasilensis (1890). Maas identified *Globba subsessilis* as an earlier synonym of *Costus warmingii* and replaced the species name with *Costus subsessilis* (Nees & Mart.) Maas (1976: 469). Subsequently, Specht and Stevenson (2006), when describing the new genus *Chamaecostus*, proposed a new combination resulting in *Chamaecostus subsessilis*, a lineage that has henceforth been treated as the *Chamaecostus subsessilis* complex.

The *C. subsessilis* species complex has been considered (Specht 2006, Maas 1972, Schumann in Engler 1904) to be closely related to *Chamaecostus cuspidatus* (Nees & Mart.) Specht & Stevenson (2006: 158), as these taxa share significant morphological similarities such as appendaged green bracts and sheaths that grow beyond the stem node, commonly covering internodes entirely. Additionally, *C. subsessilis* and *C. cuspidatus* have adjacent geographic distributions, with limited but existing range overlap in the Atlantic Rain Forest and in the Cerrado transition, Eastern Brazil. *Chamaecostus subsessilis* is a nearly acaulescent herb that inhabits the seasonally dry forests of Central South America, while *C. cuspidatus* as described is found in the Atlantic Rain Forest and is caulescent in habit.

In his comprehensive revisions of all Neotropical Costaceae, Maas (1972, 1977) included all members of the genus *Chamaecostus* as members of *Costus* subgenus *Cadalvena* (Fenzl) K.Schum. in Engler (1904: 381). However, the first family-wide investigation of the phylogenetic relationships within Costaceae (Specht *et al*. 2001) using both morphological and molecular characters revealed that *Costus* L. was paraphyletic: New world members of *Costus* subgenus *Cadalvena* group were indeed monophyletic, but more closely related to *Dimerocostus* and *Monocostus* than to other *Costus* species, rendering *Costus* paraphyletic (Specht *et al*. 2001; Specht 2006). This analysis also supported the position of the *Cadalvena* type species, *Costus spectabilis* (Fenzl) Schumann (1892: 422), with other lineages of African taxa within the genus *Costus*. Therefore, Specht & Stevenson (2006) formally elevated new world subgen. *Cadalvena* members to the genus *Chamaecostus,* with the etymology (chamae-) being indicative of their small stature (≤1 m) relative to plants remaining in the genus *Costus*. Together with *Monocostus* Schumann (1904: 427) and *Dimerocostus* Kuntze (1891: 687), these three genera form an early-diverging clade of approximately 17 species with a distribution encompassing Central and South America ("South American Clade", Specht 2006). Morphologically, *Chamaecostus*, *Monocostus* and *Dimerocostus* share cup-shaped stigmas; tubular and bicarinate bracteoles; presence of unicellular hairs; and a general flower morphology with long and narrow labellum (fused petaloid staminodes) opening into a wide and distinct limb. Additionally, *Monocostus* and *Dimerocostus* share a bilocular ovary, while the *Chamaecostus* ovary is trilocular similar to that found in *Costus*.

As their name implies, *Chamaecostus* are low plants, even occasionally emerging as acaulescent rosettes, typically not exceeding 1 m in height and with stems commonly less than 1 cm in diameter. Specht and Stevenson (2006) cite the following synapomorphies for identification of *Chamaecostus*: small stature; cup-shaped stigma; open labellum; ovary and tube of labellum red-brown punctate. Also noteworthy are their very fragile shoots and nodes that are commonly purplish and lightly geniculate. Additionally, intermittency of aerial shoots during dry season and presence of subterraneous reserve organs (tubers) are also very common. The staminodial labellum is large, ovate at the apex, yellow, orange, red, or white. Distribution of *Chamaecostus* is restricted to South America, from the Guyana Shield to the Amazonian lowlands of Bolivia and Brazil, the western edge of the Brazilian shield and the Brazilian Atlantic Rainforest. In addition, *Chamaecostus* species seem to show an aggregated distribution, with high local abundance but naturally rare occurrence along the landscape. The genus currently consists of seven known species, all endemic to South America. Geographic distribution is varied and includes seasonally dry forests of Southwest Amazonia and Cerrado forest ecosystems of Central Brazil (*Chamaecostus subsessilis*); Central Atlantic Forest (*Chamaecostus cuspidatus*); Amazonian (*Chamaecostus fusiformis* (Maas) Specht & Stevenson (2006: 158), *Chamaecostus fragilis* (Maas) Specht & Stevenson (2006: 158), *Chamaecostus lanceolatus* (Petersen) Specht & Stevenson (2006: 158)); and endemic to the Guyana shield (*Chamaecostus* congestiflorus (Rich. ex L. F. Gagnep.) Specht & Stevenson (2006: 158), *Chamaecostus curcumoides* (Maas) Specht & Stevenson (2006: 158)).

Here, we investigate the *Chamaecostus subsessilis* species complex from a population perspective. The broad geographic range of the complex and the long list of synonyms associated with taxa in this group indicate that there may be more than one species present, given a phylogenetic species concept (Nixon & Wheeler 1990). We use DNA sequences to test for monophyly of species and lineages within *Chamaecostus,* and study leaf morphometrics to test for morphological integrity of the lineages examined. Finally, we present a comprehensive molecular phylogeny of *Chamaecostus*, on which we interpret evolutionary trends within this genus.

## Material and Methods

### 2.1. Phylogenetic relationships

We analyzed the phylogenetic relationships of species within *Chamaecostus,* using Maximum Likelihood (ML; PhyML, Guidon & Gascuel 2003) and Bayesian (MrBayes, Huelsenbeck & Ronquist 2001) approaches based on a combined dataset of nuclear (ETS, Kay *et al*. 2005; ITS, White *et al*. 1990; rpb2, Specht *et al*. 2001; CaM, Salzman *et al*. 2015) and plastid (rps16-trnK, Shaw *et al*. 2007; petG-trnP, Hwang *et al*. 2000; tnrL-trnLF, Taberlet *et al*. 1991) sequences. All sequences were deposited in GenBank.

We included individuals from all known species within the genus, and designated samples of *Monocostus uniflorus,* two species of *Dimerocostus* and two species of *Costus* as outgroups. For the *Chamaecostus subsessilis* complex, we analyzed samples from 12 populations collected across its distributional range. Total genomic DNA was isolated from silica-gel dried leaf tissue using CTAB extraction protocol (Doyle & Doyle 1990). PCR fragments of the molecular markers above were generated using Phire Hot Start II DNA Polymerase (Thermo Scientific) with a 3 min. initial denaturing step at 98 °C, 45 cycles of 5 sec. at 98 °C, 15 sec. at gene-specific annealing temperatures, and 20 sec. at 72 °C, with a final 1 min. 72 °C extension. Cycle sequencing was performed using BigDye v3.1 (Applied Biosystems) following manufacture's protocols. Cycle sequencing products were sequenced on an Applied Biosystems 3730 DNA Analyzer automated DNA sequencer, at UC Berkeley's Evolutionary Genetics Laboratory.

We aligned each marker using the MUSCLE algorithm (Edgar 2004) implemented in Geneious version 6.1.7 (www.geneious.com), and subsequently checked the multiple sequence alignments manually. Sequence data was partitioned to allow different models of sequence evolution for each region, with the best model for sequence evolution determined with jModelTest version 2 (Darriba *et al*. 2012).

Maximum Likelihood (ML) analyses were run with a total of 1,000 bootstrap replicates to assess statistical clade support. Bayesian analyses were run twice for $50x10^6$ generations, sampling every 10,000 generations. Convergence was assessed via a low (<0.01) average standard deviation in split frequencies after the first 25% of the sampled data were discarded as burn-in.

### 2.2. Morphometrics of the *Chamaecostus subsessilis* complex

We measured leaves of specimens distributed across the range from the *Chamaeostus subsessilis* complex (n=134) and from the closely related species *Chamaecostus cuspidatus* (n=14) deposited in herbarium collections (A; HUFU; IAN; IBGE; INPA; MG; MO; NY; R; RB; TANG; UB; UC; UFG - acronyms follow Thiers 2014) for the assessment of the following morphometric variables: leaf length [LL], leaf maximum width [LW], apex angle [AA], base angle [BA], leaf elliptical area [$LA=\pi*((LL/2)*LW]$, leaf area eccentricity [$LE=LA/(LL/2)$], leaf length-width proportion [LL/LW], and leaf apex-base symmetry [LS=AA/BA] (Figure 1). We focused on leaf quantitative variation since vegetative characters are meaningfully variable within this group, while floral traits are reasonably constant. Only specimens with well-developed and properly pressed leaves were considered in the analysis. We also analyzed type specimens of *Chamaecostus subsessilis* synonyms (BM, K, MO, P) to review species circumscriptions.

Herbarium specimens of the *Chamaecostus subsessilis* complex were assigned to resolved phylogenetic clades based on their recorded geographic location: each herbarium specimen was assigned to the clade that contained individuals with the closest geographic proximity. Analysis of variance (ANOVA) and two-sample t-test were performed for each trait to detect differences between phylogenetic lineages given assignment to clades. Statistical analyses were computed in R framework (R Development Core Team 2014).

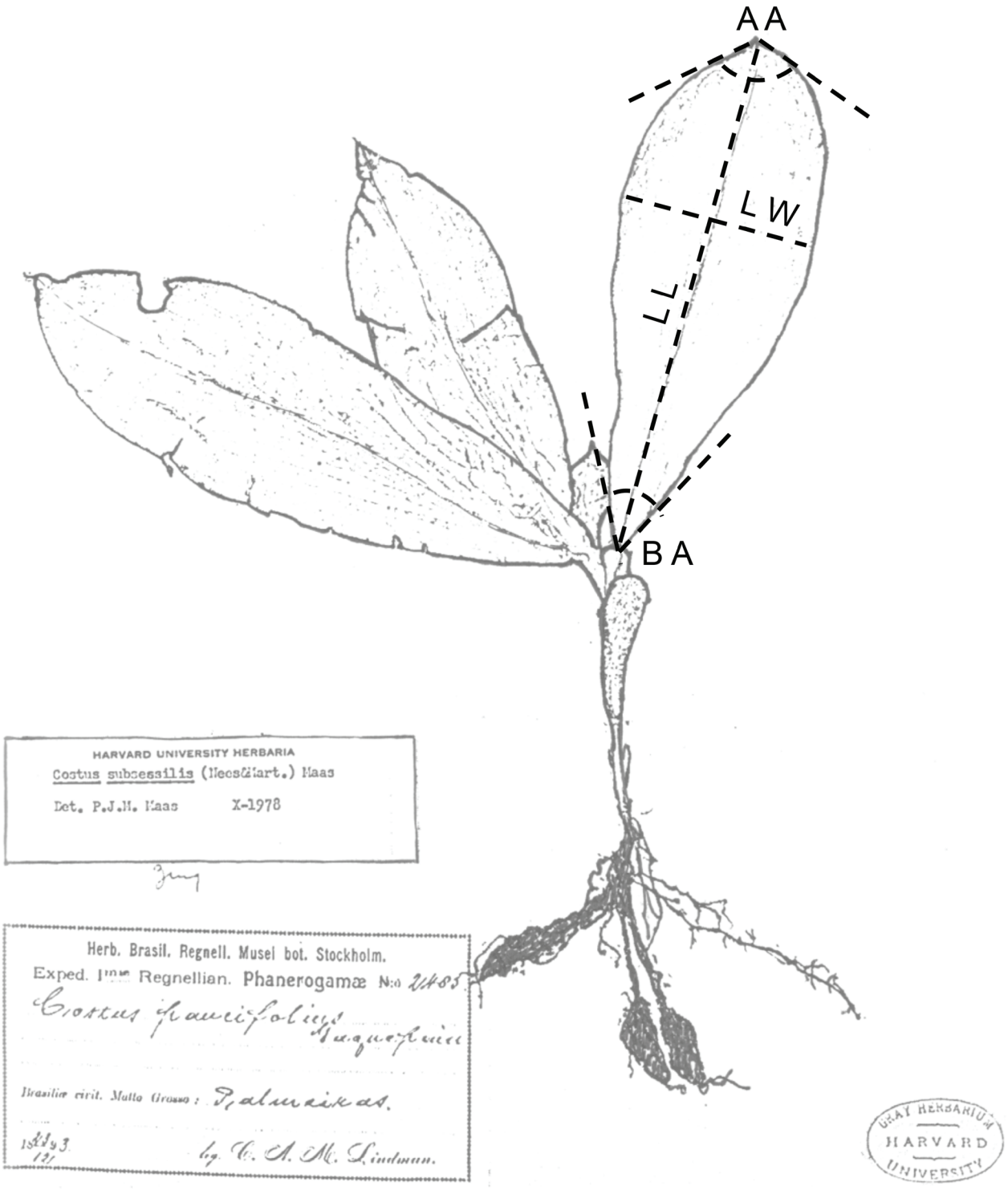


**FIGURE 1.** Schematic representation of measured morphometric variables; LL—Leaf Length, LW—Leaf Maximum Width, AA—Apex Angle, BA—Base Angle.

## Results

### 3.1. *Chamaecostus* systematics and phylogenetic relationships

The concatenated multiple sequence alignment is 3,480 base pairs long and a similar overall topology (Figure 2A) is recovered in both Maximum Likelihood (ML) and Bayesian Inference analyses. The phylogeny is well supported overall,

as shown by high node confidence provided by both Bayesian posterior probabilities and ML bootstrap replicates, with the exception of the position of *Chamaecostus fragilis*. In this analysis, *Chamaecostus subsessilis* was found to be paraphyletic. Two well-supported major clades are recovered (Figure 2A; *subsessilis* and *acaulis*), one of which is sister to *Chamaecostus cuspidatus*. These two clades correspond to previous species definitions and delimitations (see discussion below), bearing high morphological variability and overlapping characters between them.

### 3.2. Morphometrics of the *Chamaecostus subsessilis* complex

Analysis of variance and two-sample t-test detected significant morphometric differences in three leaf traits between specimens assigned to either *acaulis* (n=83) or *subsessilis* (n=51) clades: leaf length, leaf maximum width, and leaf area (Figure 3). With the exception of apex-base symmetry, all other morphometric variables significantly separate *Chamaecostus cuspidatus* from the other two clades (Table 1). In our molecular phylogeny, the *subsessilis* clade is sister to *C. cuspidatus*, but *subssessilis* and *acaulis* clades are highly similar in morphology, underscoring the significance of these quantitative differences.

**TABLE 1.** Morphometric variables tested for diagnose between *Chamaecostus cuspidatus* and the *Chamaecostus subsessilis* complex. Means ± standard deviations; different letters are indicative of statistical significance ($p<0.05$; t-test), and F-values and probabilities of ANOVA are given. Bold values refer to variables that are significantly different between all three species.

| Leaf variables | *Chamaecostus cuspidatus* | *subsessilis* clade | *acaulis* clade | ANOVA | |
|---|---|---|---|---|---|
| | (n=14) | (n=51) | (n=83) | F | p |
| Length (cm) | **14.9 ± 4.5**[a] | **18.6 ± 6.2** [b] | **22.3 ± 5.6** [c] | 13.2 | 0.000 |
| Maximum Width (cm) | **4.5 ± 1.5** [a] | **7.4 ± 2.6** [b] | **8.6 ± 2.1** [c] | 21.2 | 0.000 |
| Length-Width Proportion | 3.4 ± 0.6 [a] | 2.6 ± 0.7 [b] | 2.6 ± 0.4 [b] | 14.5 | 0.000 |
| Area ($cm^2$) | **113.4 ± 87.5** [a] | **235.7 ± 152.9** [b] | **316.0 ± 149.8** [c] | 13.5 | 0.000 |
| Eccentricity | 0.6 ± 0.1 [a] | 0.8 ± 0.2 [b] | 0.8 ± 0.1 [b] | 12.1 | 0.000 |
| Apex Angle | 84.4 ± 37.0 [a] | 44.5 ± 17.4 [b] | 78.6 ± 25.8 [b] | 10.2 | 0.000 |
| Base Angle | 30.4 ± 6.5 [a] | 53.4 ± 18.6 [b] | 52.0 ± 14.3 [b] | 13.1 | 0.000 |
| Apex-Base Symmetry | 1.5 ± 0.6 [a] | 1.7 ± 0.9 [a] | 1.6 ± 0.5 [a] | 0.7 | 0.508 |

## Discussion

### 4.1. *Chamaecostus* systematics and phylogenetic relationships

Here we present a robust molecular phylogeny of *Chamaecostus* (Figure 2A), from which novel evolutionary inferences can be inferred. Within *Chamaecostus*, major clades generally reflect relationships previously suggested from taxonomic studies (Schumann *in* Engler 1904, Maas 1972). A close relationship between *Chamaecostus curcumoides* and *Chamaecostus fusiformis* was suggested by Maas (1972) when he described both species. Indeed, the well-supported clade formed by these two species is supported morphologically by various synapomorphies of these species, such as a more complex capitate inflorescence, ovate-triangular yellow bracts, and a strongly tubular labellum. Likewise, green, appendaged bracts and a sheath longer than the corresponding node are synapomorphies of *Chamaecostus cuspidatus* and *Chamaecostus subsessilis*, a species pair whose close taxonomic relationship had been suggested previously (Nees & Martius 1823, Petersen in Martius 1890, Schumann in Engler 1904, Maas 1972). Perhaps the most remarkable implication of this *Chamaecostus* phylogeny (Figure 2A) is the paraphyly of *Chamaecostus subsessilis,* indicating the need for a revision of the genus.

Our phylogeny suggests some new interpretations and indicates alternative evolutionary scenarios with respect to biogeography and morphology. An Amazonian distribution is most likely ancestral for the genus, with the appearance in Southern and Eastern portions of South America being more derived; only *C. cuspidatus* has a distribution lying completely outside of the Amazonian domain. Also noteworthy is the position of *C. congestiflorus* as sister to the remaining species in the genus; this species has white flowers, with a conspicuous fimbriate labellum, while all other species have either a yellow, orange, red or pink glabrous labellum (at least when fully developed and opened), suggesting that a shift from white flowers and a reduction of the labellum margin complexity evolved only once in the genus leading to a radiation of colorful-flowered species with a glabrous labellum.

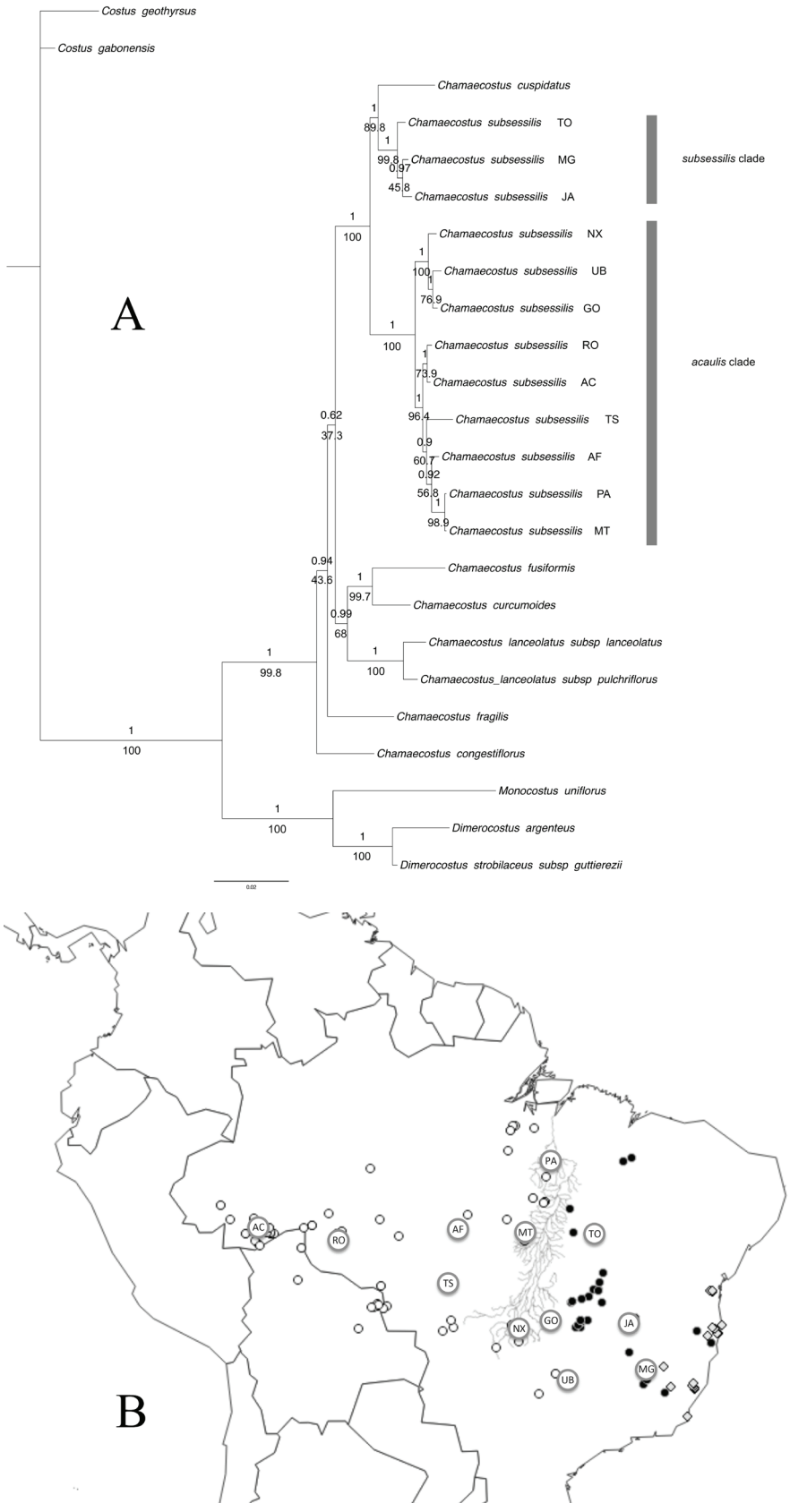


**FIGURE 2.** A—Phylogenetic relationships within *Chamaecostus* (Costaceae). Support values above branches are Bayesian Posterior Probabilities, while Most Likely Bootstrap proportions from 1,000 bootstrap replicates are found below branches; B—Geographic ranges of the *Chamaecostus cuspidatus* (diamonds), and *Chamaecostus subsessilis* complex: *subsessilis* clade (solid circles) and *acaulis* clade (open circles). The continuous grey line identifies the Araguaia River.

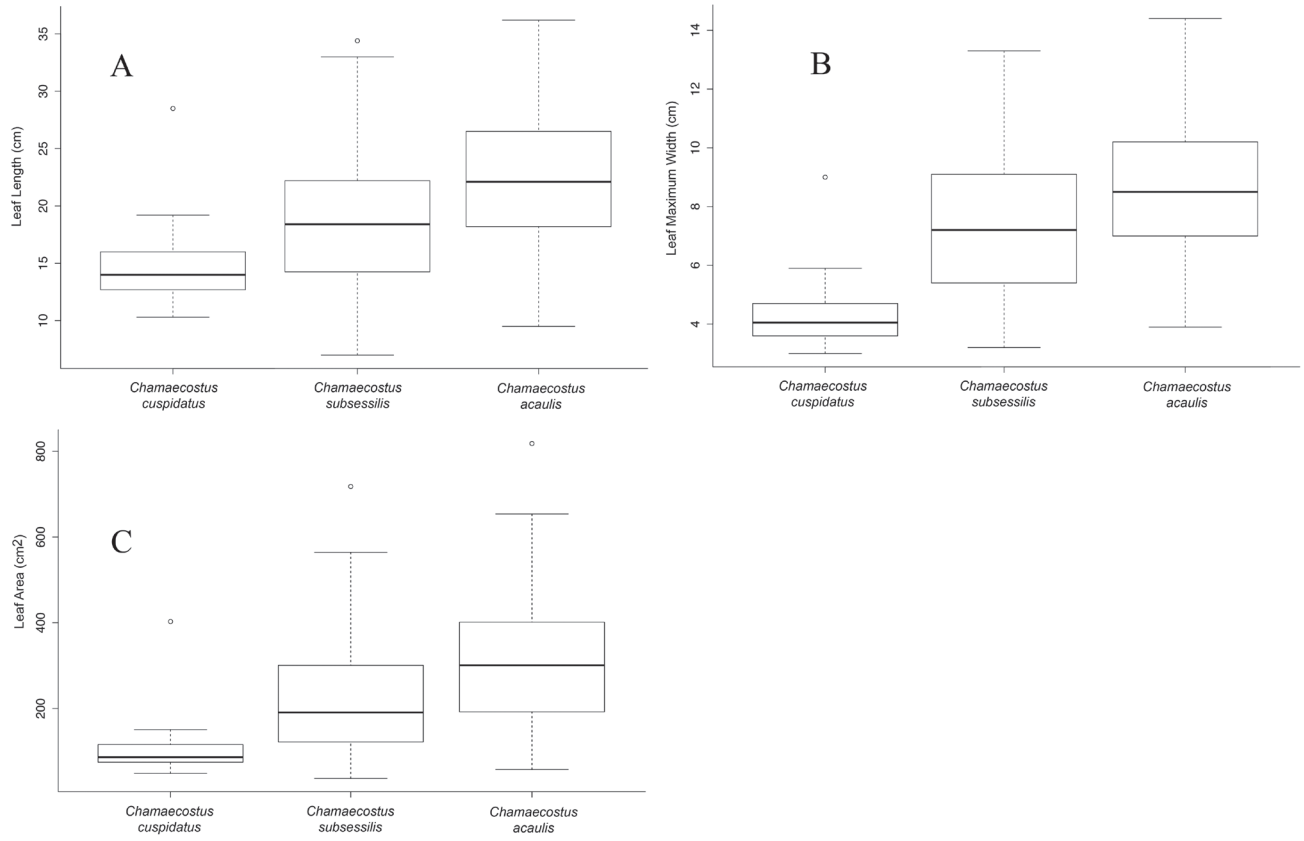


**FIGURE 3.** Box and whisker plots of three significantly different morphometric variables between *Chamaecostus cuspidatus* (n=14), *Chamaecostus subsessilis* s.str. (n=51) and *Chamaecostus acaulis comb. nov.* (n=83), showing means, quartiles and ranges. A—Leaf length (cm); B—Leaf Maximum Width (cm); C—Leaf Area ($cm^2$).

### 4.2. Morphometrics of the *Chamaecostus subsessilis* complex

Redefining species limits in the *Chamaecostus subsessilis* complex can be rather problematic, particularly because of the limited extent of morphological diagnostic characters, especially when considering morphological differences from every other species in the genus, which can be exceptionally divergent. Indeed, distinct species criteria describe different stages in the divergence of lineages, and differences among the many species concepts are at least partly attributable to the complex and temporally extended nature of speciation (de Queiroz 2007), and therefore no single definition will be appropriate for all organisms. Morphological similarity has the disadvantage of using arbitrary determinations of the threshold of differences (de Queiroz 1998) and is especially challenging to apply when quantitative character continua are significant. Such is indicated to be the case for the *C. subsessilis* complex, where character variation among and between populations was thought to be solely due to environment (Maas 1972,1977). Nevertheless, determining these two distinct *C. subsessilis* lineages to be a single species in face of the strong population genetic structure would be unfitting and would not reflect the evolutionary differentiation among sampled populations condition.

Results do show significant morphometric size differences between the two lineages (Table 1, Figure 3). Additionally, *a posteriori* interpretation of the descriptions of *Chamaecostus subsessilis* synonyms from the historic record also reveals traits that are useful to separate these lineages. Petersen (in Martius' Flora Brasiliensis 1890) describes a *Costus warmingii* as being over 1 m tall with elliptic to obovate-elliptic leaves, variable in size, abaxially densely hirsute and adaxially glabrous. Later, Schumann (in Engler 1904) mentioned other individuals under the synonyms *Costus gagnepainii* K.Schum. in Engler (1904: 420), *Costus latifolius* Gagnepain (1902: 100), *Costus paucifolius* Gagnepain (1902: 100), *Costus pumilus* Petersen in Martius (1890: 58), and *Costus rosulifer* Gagnepain (1902: 101), as having either both or at least one leaf side glabrous. All of the types for the synonyms occur East of the Araguaia River valley. Moore (1895: 480) described *Costus acaulis* (=*Chamaecostus subsessilis*) as having oblong-obovate puberulous leaves based on a type from Mato Grosso, in the upper Paraguay River basin, Brazil. This specimen greatly reassembles Loesener's (1929) descriptions and types for *Costus steinbachii* Loesener (1929: 714) and *Costus kaempferoides* Loesener (1929: 714), from Bolivia and Peru, respectively. Interestingly, Specht

(2006) noted that although indument characters in general tend to be homoplasious in Costaceae, certain aspects of the indument do help to define some lineages.

Hence, external morphology, anatomy, ecology, life history and reproductive biology should be further investigated in a finer and more detailed fashion to help accumulate diagnostic features for *Chamaecostus* species, with a specific focus on detecting synapomorphies between *C. cuspidatus* and *C. subsessilis* s.str. (*subsessilis* clade in Figure 2A), since compilation of relevant information often leads to an improved comprehension of boundaries between species (e.g. Wendt *et al*. 2011, Faria *et al*. 2010). One or only a few individuals may not be representative of the species as a whole, especially for taxa with widespread distributions (Goldstein *et al.* 2000; Walsh 2000). An integrative approach, combining population genetics, historical biogeography, and environmental data, could be of great help to elucidate the speciation scenario and demographic history involved. Our inspection of several individuals from multiple localities across the range of the species provides discernible differences between clades, highlighting the particular efficacy of population sampling to fully determine species integrity. Thus, we encourage broad geographic and genetic sampling to investigate the possibility of cryptic evolutionary lineages in species complexes.

Species polyphyly can result as an artifact of phylogenetic reconstruction from weak phylogenetic signal or incomplete lineage sorting, or from taxonomically underestimating or overestimating genetic exchange among individuals and populations (Funk & Omland 2003). Furthermore, the use of morphological or genetic markers alone could mislead our understanding of evolutionary history; phylogeographic breaks can form within a continuously distributed species even when there are no barriers to gene flow if the average individual dispersal distance and local population size are small (Irwin 2002). Correspondingly, morphometric data commonly convey ontogenetically or ecologically governed plasticity and could obscure genetically governed morphological variation relevant to taxonomic decisions (Tetsana *et al.* 2014). Our comprehensive approach, combining phylogenetic analysis of multiple molecular markers and leaf morphometrics, objectively reveals the recognition of at least two species within the *Chamaecostus subsessilis* complex as necessary to appropriately reflect evolutionary relationships, and we formally acknowledge distinct names below.

## Taxonomy

*Chamaecostus acaulis* (S.Moore) T.André & C.D.Specht ***comb. nov.*** = *Costus acaulis* S.Moore. Transactions of the Linnean Society of London 4: 480, pl. 33, f. 1–5. 1895; Type:—BRAZIL. Mato Grosso: Santa Cruz (i.e. Barra do Bugres), November 1891, Spencer Moore 679 (holotype, BM!).

= *Costus steinbachii* Leoesener, Notizbl. Bot. Gart. Berl. 10: 714. 1929; Loesener in Engler & Prantl, Nat. Pflanzenfam. ed. 2. 15A: 634. 1930. Type:—BOLIVIA. Sara: Santa Cruz, 31 December 1925, Steinbach 7386 (lectotype, F; isolectotypes, K!, MO!). Lectotype assigned by Maas (1972) since the holotype was destroyed at Berlin in 1943.

= *Costus kaempferoides* Leoesener, Notizbl. Bot. Gart. Berl. 10: 714. 1929. Type:—PERU. Madre de Dios: Seringal São Francisco, September 1911, Ule 9197 (lectotype, K!). Lectotype assigned by Maas (1972) since the holotype was destroyed at Berlin in 1943.

Acaulescent or very low plants with stems up to 30 cm long and 1–10 mm wide; Internode 1.0–8.5 (3.5 ± 1.6) cm long; Roots fleshy, with tubers; root tubers fusiform to ellipsoid; Sheaths membranaceous, 1.5–3.0 (1.6 ± 0.6) cm long, 1.5–8.5 (4.5 ± 1.7) cm wide, obtuse at the apex, puberulous. Ligule 1 mm long. Leaves (4–6), rosulate, elongate, oblong-obovate, 22.3 ± 5.6 cm long, 8.6 ± 2.1 cm wide, densely strigose or densely to sparsely puberulous on both sides, cuneate at the base, shortly acuminate at the apex, apex up to 2.0 cm (0.6 ± 0.4), margins densely ciliate; Inflorescence compact, terminal and short; bracts herbaceous, green to 30–70 (50 ± 30) mm long, 5–15 (10 ± 5) mm wide, densely puberulous; appendages foliaceous, green, narrowly triangular to deltate, mucronate at the apex, densely puberulous. Bracteole membranaceous, tubular, 2–3 cm long. Calyx tubular, membranaceous to herbaceous, 10–40 mm long, lobes narrowly triangular, mucronate, 1–15 mm long. Corolla white, 50–70 mm long, tube 25–30 mm long, lobes narrowly elliptic, mucronate, 30–40 mm long, 6–12 mm wide. Labellum yellow, with white, yellow or orange nectar guides at the middle, broadly obovate, 60–70 mm long, 70–95 mm wide, margins undulate, lightly fimbriate or glabrous. Stamen yellow, oblong-oblanceolate, Stamen 20–50 mm long, up to 20 mm wide, apex obtuse, irregularly lobed or acuminate, anther attached at the base, caudate at the apex. Style filiform, glabrous; Stigma cup-shaped; Fruits and seeds not analyzed.

*Chamaecostus acaulis* strongly resembles *Chamaecostus subsessilis* sensu stricto, but differs by developing

shorter habit, bigger oblong-obovate leaves (22.3 ± 5.6 cm long x 8.6 ± 2.1 cm wide), and by possessing puberulous leaves. *Chamaecostus subsessilis* sensu stricto have adaxially strigose to glabrous leaves, more elliptical and smaller leaves (18.6 ± 6.2 cm long x 7.4 ± 2.6 cm wide), and variable height, from 0.3 to over 1.0 m.

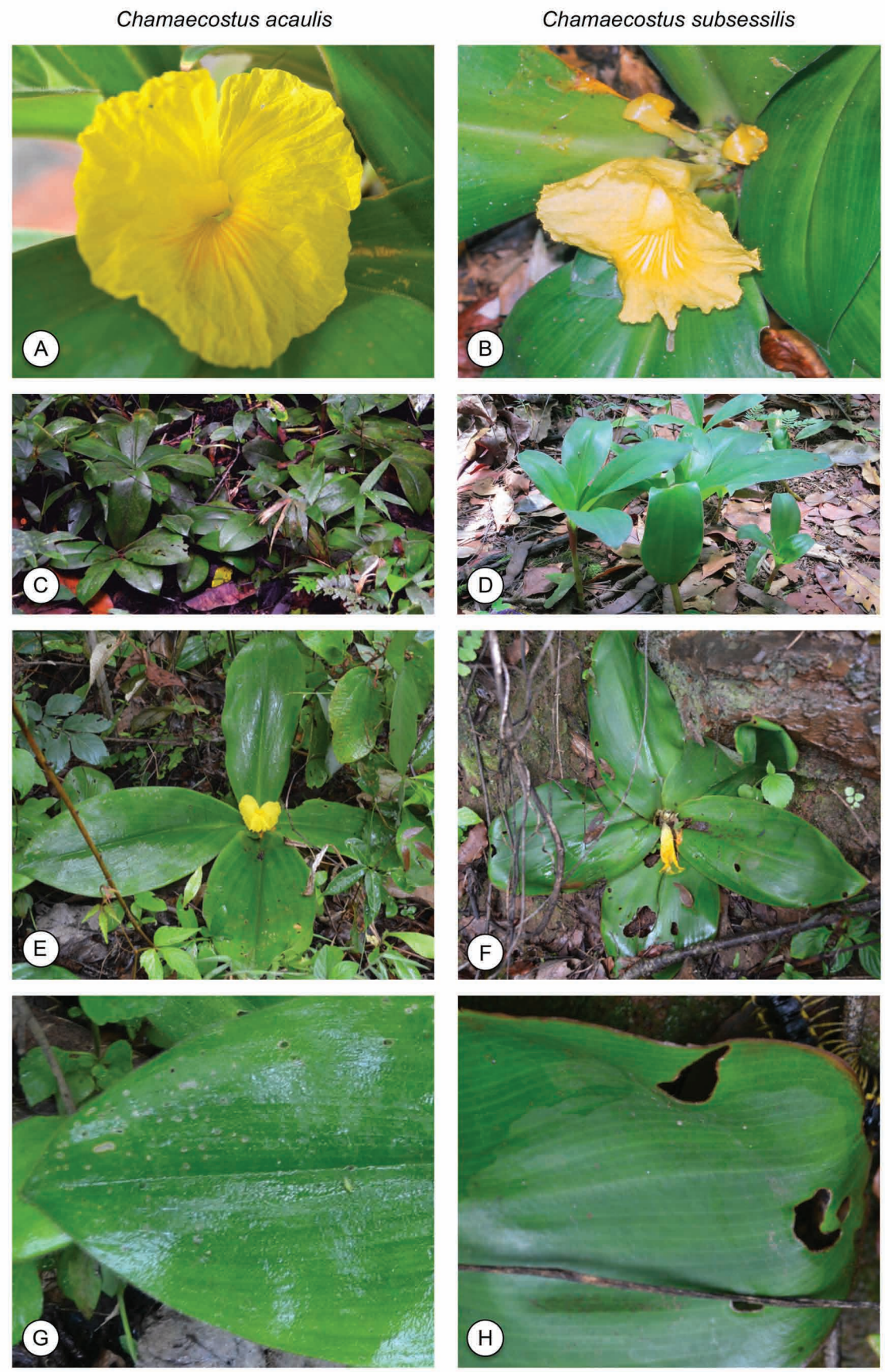


**FIGURE 4.** *Chamaecostus acaulis comb. nov.* and *Chamaecostus subsessilis* s.str.. (B) photo by W.W.Thomas. (D) photo by D.Skinner.

Since there is substantial overlap in characters between the two (Figure 3, Table 1), we strongly suggest that location should be taken into account when identifying both species, in particular because of the strong geographic structure resolved between the populations analyzed here. *Chamaecostus acaulis* occurs West of the Araguaia River valley, through Peruvian, Bolivian and Brazilian South Amazonia, and in Western and Southern portions of the Central Brazilian Shield, while *Chamaecostus subsessilis* occurs East from the Araguaia River valley, and within most of the Central Brazilian Shield and in transition forests between Cerrado and Central Atlantic Rain Forest (Figure 2B). However, potentially sympatric populations may occur within the Araguaia River valley, and further detailed analyses of the populations in this transition zone are necessary.

## Acknowledgments

We are thankful to visited herbaria curators and staff. This paper is part of the D.Sc. requirements of TA at the Biodiversity and Evolutionary Biology Graduate Program of the Federal University of Rio de Janeiro. TA received a scholarship grant from *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*. TW received a productivity grant from *Conselho Nacional de Desenvolvimento Científico e Tecnológico*. CS received support from the US National Geographic Society (CRE Grant #8994-11) and CPS received support from FAPESP (2009/52725-3), that helped support this research.

## References

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.
http://dx.doi.org/10.1038/nmeth.2109

de Queiroz, K. (1998) The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. *In:* Howard, D.J. & Berlocher, S.H. (Eds.) *Endless forms: Species and speciation* Oxford University Press, New York, pp. 57–75.

de Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology* 56: 879–886.
http://dx.doi.org/10.1080/10635150701701083

DeSalle, R., Egan, M., Sidall, M. (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B* 360: 1905–1916.
http://dx.doi.org/10.1098/rstb.2005.1722

Doyle, J.J. & Doyle, J.L. (1990) A rapid total DNA preparation procedure for fresh plant tissue. *Focus* 12:13–15.

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32:1792–1797.
http://dx.doi.org/10.1093/nar/gkh340

Engler, H.G.A. (Ed.) (1904) *Pflanzenreich IV*. 46 (Heft 20).

Faria, A.P.G., Wendt, T., Brown, G.K. (2010) A revision of *Aechmea* subgenus *Macrochordion* (Bromeliaceae) based on phenetic analyses of morphological variation. *Botanical Journal of the Linnean Society* 162: 1–27.
http://dx.doi.org/10.1111/j.1095-8339.2009.01019.x

Florio, A.M., Ingram, C.M., Rakotondravony, H.A., Louis, Jr. E.E. & Raxworthy, C.J. (2012) Detecting cryptic speciation in the widespread and morphologically conservative carpet chameleon (*Furcifer lateralis*) of Madagascar. *Journal of Evolutionary Biology* 25: 1399–1414.
http://dx.doi.org/10.1111/j.1420-9101.2012.02528.x

Frost, D.R. & Kluge, A.G. (1994) A consideration of epistemology in systematic biology, with special reference to species. *Cladistics* 10: 259–294.
http://dx.doi.org/10.1111/j.1096-0031.1994.tb00178.x

Funk, D.J. & Omland, K.E. (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics* 34: 397–423.
http://dx.doi.org/10.1146/annurev.ecolsys.34.011802.132421

Gagnepain, F. (1902) *Bulletin de la Société Botanique de France*, *Series 4* 2: 102–103.

Goldstein, P.Z. & DeSalle, R. (2000) Phylogenetic species, nested hierarchies, and character fixation. *Cladistics* 16: 364–384.
http://dx.doi.org/10.1111/j.1096-0031.2000.tb00356.x

Goldstein, P.Z., DeSalle, R., Amato, G. & Vogler, A.P. (2000) Conservation genetics and the species boundary. *Conservation Biology* 14: 120–131.
http://dx.doi.org/10.1046/j.1523-1739.2000.98122.x

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic*

*Biology* 52: 696–704.
http://dx.doi.org/10.1080/10635150390235520
Hey, J. & Pinho, C. (2012) Population genetics and objectivity in species diagnosis. *Evolution* 66: 1413–1429.
http://dx.doi.org/10.1111/j.1558-5646.2011.01542.x
Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
http://dx.doi.org/10.1093/bioinformatics/17.8.754
Hwang, L.H., Hwang, S.Y. & Lin, T.P. (2000) Low chloroplast DNA variation and population differentiation of Chamaecyparis formosensis and Chamaecyparis taiwanensis. *Taiwan Journal of Forest Science* 15: 229–236.
Irwin, D.E. (2002) Phylogeographic breaks without geographic barriers to gene flow. *Evolution* 56: 2383–2394.
http://dx.doi.org/10.1554/0014-3820(2002)056[2383:PBWGBT]2.0.CO;2
Kay, K.M., Reeves, P.A., Olmstead, R.G. & Schemske, D.W. (2005) Rapid speciation and the evolution of hummingbird pollination in neotropical *Costus* subgenus Costus (Costaceae): evidence from nrDNA ITS and ETS sequences. *American Journal of Botany* 92: 1899–1910.
http://dx.doi.org/10.3732/ajb.92.11.1899
Kuntze, C.E.O. (1891) *Revisio Generum Plantarum* 2: 687.
Linnaeus, C. (1753) *Species Plantarum* 1: 1–560.
Loesener, L.E.T. (1929) *Notizblatt des Botanischen Gartens und Museums zu Berlin-Dahlem* 10: 714.
http://dx.doi.org/10.2307/3994705
Maas, P.J.M. (1972) Costoideae (Zingiberaceae). *In: Flora Neotropica*. *Monograph 8.* Hafner, New York, New York, USA.
Maas, P.J.M. (1976) Notes on New World Zingiberaceae. *Acta Botanica Neerlandica* 24: 469.
Maas, P.J.M. (1977) *Renealmia* (Zingiberaceae-Zingiberoideae) Costoideae (Additions) (Zingiberaceae). *Flora Neotropica*. Monograph 18. Hafner, New York, New York, USA.
Martius, C.F.P. (Ed.) (1890) *Flora Brasiliensis*. Vol III. Pars III, pp. 1–128.
Moore, S. (1895) *Transactions of the Linnean Society Series 2* 4: 480.
Nees, C.G.D. & Martius, C.F.P. (1823) *Nova Acta Physico-medica Academiae Caesareae Leopoldino-Carolinae Naturae Curiosorum Exhibentia Ephemerides sive Observationes Historias et Experimenta* 11: 29.
Nixon, K.C., Wheeler, Q.D. (1990) An amplification of the phylogenetic species concept. *Cladistics* 6: 211–223.
http://dx.doi.org/10.1111/j.1096-0031.1990.tb00541.x
Padial, J. & De La Riva, I. (2009) Integrative taxonomy reveals cryptic Amazonian species of Pristimantis (Anura: Strabomantidae). *Zoological Journal of the Linnean Society* 155: 97–122.
http://dx.doi.org/10.1111/j.1096-3642.2008.00424.x
Padial, J., Miralles, A., De la Riva, I. & Vences, M. (2010) The integrative future of taxonomy. *Frontiers in Zoology* 7: 16.
http://dx.doi.org/10.1186/1742-9994-7-16
R Development Core Team (2014) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Available form: http://www.R-project.org.
Rieseberg, L.H., Church, S.A. & Morjan, C.L. (2004) Integration of populations and differentiation of species. *New Phytologist* 161: 59–69.
http://dx.doi.org/10.1046/j.1469-8137.2003.00933.x
Sáez, A.G. & Lozano, E. (2005) Body doubles. *Nature* 433:111.
http://dx.doi.org/10.1038/433111a
Salzman, S., Driscoll, H.E., Renner, T., André, T., Shen, S. & Specht, C.D. (2015) Spiraling into history: A molecular phylogeny and investigation of biogeographic origins and floral evolution for the genus *Costus*. *Systematic Botany* 40: 104–115.
http://dx.doi.org/10.1600/036364415X686404
Schumann, K. (1892) *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 15: 422.
http://dx.doi.org/10.1080/10635150701772563
Shaffer, H.B. & Thomson, R.C. (2007) Delimiting Species in Recent Radiations. *Systematic Biology* 56: 896–906.
Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in Angiosperms: the tortoise and the hare III. *American Journal of Botany* 94: 275–288.
http://dx.doi.org/10.3732/ajb.94.3.275
Specht, C.D., Kress, W.J., Stevenson, D.W. & DeSalle, R. (2001) A Molecular Phylogeny of Costaceae (Zingiberales). *Molecular Phylogenetics and Evolution* 21: 333–345.
http://dx.doi.org/10.1006/mpev.2001.1029
Specht, C.D. & Stevenson, D.W. (2006) A new phylogeny-based generic classification of Costaceae (Zingiberales). *Taxon* 55:153–163.
http://dx.doi.org/10.2307/25065537
Specht, C.D. (2006) Systematics and Evolution of the Tropical Monocot Family Costaceae (Zingiberales): A Multiple Dataset Approach. *Systematic Botany* 31: 89–106.
http://dx.doi.org/10.1600/036364406775971840
Taberlet, P, Gielly, L, Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
http://dx.doi.org/10.1007/BF00037152

Tetsana, N., Pedersen, H.Æ. & Sridith, K. (2014) Character intercorrelation and the potential role of phenotypic plasticity in orchids: a case study of the epiphyte Liparis resupinata. *Plant Systematics and Evolution* 300: 517–526 http://dx.doi.org/10.1007/s00606-013-0900-0

Thiers, B. (2014) *Index Herbariorum*: A global directory of public herbaria and associated staff. *New York Botanical Garden's Virtual Herbarium*. Available from: http://sweetgum.nybg.org/ih/.

Walsh, P. (2000) Sample size for the diagnosis of conservationunits. *Conservation Biology* 14: 1533–1535. http://dx.doi.org/10.1046/j.1523-1739.2000.98149.x

Wendt, T, da Cruz, D.D., Demuner, V.G., Guilherme, F.A.G. & Boudet-Fernandes, H. (2011) An evaluation of the species boundaries of two putative taxonomic entities of *Euterpe* (Arecaceae) based on reproductive and morphological features. *Flora* 206: 144–150. http://dx.doi.org/10.1016/j.flora.2010.03.002

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In: PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

## Supplementary Information: Measured specimens

*Chamaecostus acaulis*. **BRAZIL**. ACRE. Porto Acre: *Lowrie 526* (INPA, MG, NY); Rio Branco: *Albuquerque 1389* (NY), *Cid 2806* (INPA, NY), *Ehrich 8* (NY), *Lowrie 445* (INPA, R); Santa Rosa: *Daly 11103* (NY); Sena Madureira: *Daly 7855* (NY), *Daly 7860* (NY), *Prance 7623* (MO), *Ramos 682* (INPA); Tarauacá: *Prance 7416* (INPA, NY); Xapuri: *Alves 2365* (NY), *Daly 7195* (MO, NY). GOIÁS. Cabeceiras: *Irwin 10358* (MO); Caipônia: *Prance 59644* (A, NY); Cidade de Goiás: *Kirkbridge 3399* (UB); Santa Rita do Araguaia: *Rocha sn* (UB). MATO GROSSO. Alta Floresta: *André 804* (RB), *Richter 39* (RB); Aripuanã: *Berg 18519* (INPA, NY); Cuiabá: *Andersson 1623* (A, UB); Barra do Garças: *Philcox 4000* (UB); Palmeiras: *Lindman 2485* (A); Poconé: *Maciel 144* (INPA); Santa Teresinha: *Oliveira 3094* (RB), *Thomas 4384* (INPA, MG). Tangará da Serra: *Silva 498* (TANG). MINAS GERAIS. Ituitaba: *Macedo 1316* (RB), *Macedo 1993* (MO); Uberlândia: *Arantes 1124* (HUFU), *Barbosa 281* (HUFU). PARÁ. Altamira: *Balée 1996* (NY), *Dias 1109* (MG), *Ferreira 1068* (NY), *Lima 6020* (RB), *Nascimento 1177* (NY), *Souza 1068* (MG), *Souza 1177* (MG); Conceição do Araguaia: *Plowman 8448* (A, INPA, MG, MO, NY); Nova Canaã dos Carajás: *Lobato 2596* (MG); Redenção: *Cordeiro 2851* (IAN); Serra do Cachimbo: *Prance 25218* (MG). RONDÔNIA. Abunã: *Prance 8338* (INPA, MG, NY); Ariquemes: *Zarucchi 2677* (A, INPA, MG, MO, NY, R, RB), *Mota 440* (NY), *Vieira 440* (MO, R), *Vieira 443* (INPA); Mutumparaná: *Prance 8975* (A, INPA, MG, NY, R). **BOLIVIA**. Bela Vista: *Steinbach 7386* (MO); Beni: *Maas 8660* (MO, NY, RB), *Surubi 313* (NY), *Rusby 1399* (A, NY), *Ledezma 893* (MO); Ñuflo de Chaves: *Ortiz 39* (NY); Santa Cruz: *Arroyo sn* (MO), *Carrión 506* (MO), *Castro 60* (MO), *Garvizu 513* (MO), *Guillén 3035* (MO), *Guillén 3623* (MO), *Killeen 7168* (MO), *Mamani 1091* (MO), *Quevedo 2473* (MO), *Rodriguez 572* (MO).

*Chamaecostus subsessilis* s.str.. **BRAZIL**. BAHIA. Itamaraju: *Mori 10753* (NY, RB); Jussari: *Belém 2274* (UB), *Thomas 11937* (MO), *Thomas 13401* (MO, NY). DISTRITO FEDERAL. Brasilia: *Barroso 639* (RB), *Heringer 10751* (IAN, UB), *Irwin 19440* (NY, RB, UB), *Pereira 2251* (IBGE, RB), *Pires 51* (RB), *Pires 57147* (UB). GOIÁS. Alto Paraíso: *Felfili 379* (IBGE), *Mendonça 2898* (IBGE); Alvorada do Norte: *Hatschbach 39011* (NY, UC); Caldas Novas: *Vieira 1652* (RB); Catalão *Hatschbach 55820* (MO); Cocalzinho: *Mendonça 2206* (IBGE); Corumbá: *Maguire 57147* (MG, MO); Formosa: *Irwin 9066* (UB); Goiânia: *Rizzo 2566* (UFG), *Rizzo 12305* (UFG); Inhumas: *Rizzo 2779* (UFG); Luziânia: *Coradin 7397* (RB); Monte Alegre: *Mendonça 4512* (RB); Mossâmedes: *Forzza 2500* (RB); Niquelândia: *Cordovil 106* (RB), *Fonseca 1245* (IBGE, UFG); Nova Roma: *Forzza 2541* (RB); São Domingo: *Oliveira 1117* (IBGE), *Santos 2367* (RB); Trindade: *Rizzo 3113* (UFG). MARANHÃO. Tuntum: *Santos 707* (MG, NY). MINAS GERAIS. Abre Caminho: *Pereira 59* (RB); Jacinto: *Leitman 51* (RB); Januária: *Filgueiras 1950* (IBGE), *Ratter 2630* (IAN, NY, UB, RB), *Ratter 6410* (IBGE); Lagoa Santa: *Hoehne 6206* (R); Minas: *Duarte sn* (RB); Serra do Cipó: *Heringer 7343* (UB); Unaí: *Brina sn* (RB); Várzea da Palma: *Duarte 7547* (RB). TOCANTINS. Aurora do Norte: *Pereira 2009* (IBGE); Lajeado: *Árbocz 6293* (IBGE); Presidente Kennedy: *Plowman sn* (INPA).

*Chamaecostus cuspidatus*. **BRAZIL**. BAHIA. Belmonte: *Mattos 368* (NY), Mattos 1804 (NY), *Santos 828* (RB); Eunápolis: *Santos 893* (NY, RB), *Mello Filho 2980* (R); Gandú: *Santos 1157* (NY, UB); Porto Seguro: *Duarte 5668* (RB), *Pinheiro 1747* (RB); Wanceslau Guimarães: *Thomas 9329* (MO). ESPÍRITO SANTO. Colatina: *Kuhlmann 6660* (RB); Santa Teresa: *Boone 984* (MO).