

Genetic relationships among *Heliconia* (Heliconiaceae) species based on RAPD markers

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ABSTRACT. The family Heliconiaceae contains a single genus, *Heliconia*, with approximately 180 species of Neotropical origin. This genus was formerly allocated to the family Musaceae, but today forms its own family, in the order Zingiberales. The combination of inverted flowers, a single staminode and drupe fruits is an exclusive characteristic of *Heliconia*. Heliconias are cultivated as ornamental garden plants, and are of increasing importance as cut flowers. However, there are taxonomic confusions and uncertainties about the number of species and the relationships among them. Molecular studies are therefore necessary for better understanding of the species boundaries of these plants. We examined the genetic variability and the phylogenetic relationships of 124 accessions of the genus *Heliconia* based on RAPD markers. Phenetic and cladistic analyses, using 231 polymorphic RAPD markers, demonstrated that the genus *Heliconia* is monophyletic. Groupings corresponding to currently recognized species and some subgenera were found, and cultivars and hybrids were found to cluster with their parents. RAPD analysis generally agreed with morphological species classification, except for the position of the subgenus *Stenochlamys*, which was found to be polyphyletic.

Key words: Genetic variability; *Heliconia*; RAPD; Molecular marker; Phylogenetic relationships; Zingiberales

INTRODUCTION

The Heliconiaceae family contains a single genus, *Heliconia* L., with approximately 200 to 250 species of Neotropical origin, ranging from north Mexico to the south of Brazil. Only a small paleotropical group, with approximately six species, is endemic to the Pacific Islands (Berry and Kress, 1991; Andersson, 1998). In Brazil, there are about 40 species distributed in two main areas, the Amazon Basin and the Atlantic Forest, which correspond to the primary areas of the distribution of the genus in the country (Kress, 1990).

Originally, heliconias were included in the family Musaceae, but the genus was always considered to be homogeneous and with its own characteristics, such as inverted flowers, the presence of a single staminode and drupe-type fruits. Nakai (1941) raised *Heliconia* to the family level (Heliconiaceae), and today, this family has only one genus (*Heliconia*), belonging to the order Zingiberales, which comprises eight families: Musaceae (bananas), Strelitziaceae (the birds of paradise), Lowiaceae (no common name), Heliconiaceae (heliconias), Zingiberaceae (the gingers), Costaceae (the costus), Cannaceae (the cannas), and Marantaceae (the prayer plants) (Berry and Kress, 1991).

Heliconias are herbaceous erect perennial plants, with simpodial rhizomes (Cronquist, 1981), and possess a pseudocaulis formed by the juxtaposition of the petioles or leaf laminas, with heights varying from less than 1 to 7 m, depending on the species (Dahlgren et al., 1985). The leaves are distichous, with a long basal sheath and a long and expanded petiole (Cronquist, 1981).

The inflorescence is terminal, erect or pendant, composed of bracts in one plane (distichous) or spirally arranged. Each bract constitutes and involves one cincinnus with many flowers. The bracts are modified leaves, cymbiform or lanceolate-conduplicate, with variable coloration, size, arrangement, texture, and number, and some of these characteristics are used in the subgenus classification (Cronquist, 1981; Berry and Kress, 1991; Andersson, 1992). Nowadays, heliconias may be subdivided into five subgenera: *Taeniostrobos* (Kuntze) Griggs; *Heliconia* (Andersson, 1981, 1985, 1992); *Stenochlamys* Baker; *Griggsia* L. Andersson, and *Heliconiopsis* (Miq.) Kress, which contains the Pacific Islands species.

The genus *Heliconia* L. contains a great diversity of species, varieties, hybrids, and cultivars of ornamental and commercial interest. However, there is confusion and uncertainty about the number of species and the relationships among them. Therefore, molecular studies may help to increase our understanding of the genetic variability in the genus and its speciation process.

RAPD (random amplified polymorphic DNA) (Williams et al., 1990) markers have been applied in genetic variability studies of many plants, such as in tree species (Ciampi and Magalhães, 2001), *Oryza* (Buso et al., 1998) and *Capsicum* (Buso et al., 2003). RAPD markers were also shown to be a powerful tool for genetic variability studies and clarification of the relationship between *Heliconia* species (Kumar et al., 1998). The objective of the present study was to use RAPD markers to further analyze the genetic variability and phylogenetic relationships among *Heliconia* species, cultivars and hybrids.

MATERIAL AND METHODS

One hundred and twenty-four accessions were analyzed, composed of 119 *Heliconia* species, cultivars and hybrids (Table 1) and five accessions from other genera of the order Zingiberales (Table 2), which were used to root the dendrograms. DNA was extracted from fresh leaves using a CTAB protocol (Doyle and Doyle, 1987), quantified on agarose gels, and diluted to a final concentration of approximately 3.0 ng/ μ L. One hundred and fifty different 10-mer RAPD primers (Operon Technologies Inc.) were screened for DNA amplification and polymorphic fragment quantity and quality. The markers were selected based on their polymorphism and robustness.

Table 1. List of the 119 accessions of *Heliconia* and reference to the materials deposited in the Brasília University Herbarium.

Taxons	Voucher specimens
Subgenus <i>Heliconia</i>	
<i>H. episcopalis</i> Vell.	
<i>H. bihai</i> (L.) L.	
<i>H. bihai</i> (L.) L.	
<i>H. bihai</i> (L.) L. cv. Banana Split	Marouelli, L.P. 7 (UB)
<i>H. bihai</i> (L.) L. cv. Nappi Yellow	
<i>H. bihai</i> (L.) L. cv. Nappi Yellow	Marouelli, L.P. 26 (UB)
<i>H. bihai</i> (L.) L. cv. Lobster Claw Two	
<i>H. bihai</i> (L.) L. cv. Lobster Claw Two	Marouelli, L.P. 3 (UB)
<i>H. bihai</i> (L.) L. cv. Orange Peachy	Marouelli, L.P. 33 (UB)
<i>H. bihai</i> (L.) L. (Terra do Meio)	
<i>H. bihai</i> (L.) L. cv. Kamehameha	Marouelli, L.P. 47 (UB)
<i>H. bihai</i> (L.) L. cv. Emerald Forest	
<i>H. bourgaeana</i> Petersen	Marouelli, L.P. 25 (UB)
<i>H. caribea</i> Lamarck	
<i>H. caribea</i> Lamarck	
<i>H. caribea</i> Lamarck	Marouelli, L.P. 35 (UB)
<i>H. champneiana</i> Griggs	
<i>H. champneiana</i> Griggs cv. Splash	
<i>H. orthotricha</i> L. Anders. cv. Eclipse Total	
<i>H. orthotricha</i> L. Anders. cv. Eclipse Total	Marouelli, L.P. 48 (UB)
<i>H. stricta</i> Huber	
<i>H. stricta</i> Huber cv. Dwarf Jamaica	
<i>H. stricta</i> Huber cv. Dwarf Jamaica	Marouelli, L.P. 15 (UB)
<i>H. stricta</i> Huber cv. Fire Bird	
<i>H. stricta</i> Huber cv. Tagami	Marouelli, L.P. 27 (UB)
<i>H. stricta</i> Huber cv. Tagami	
<i>H. stricta</i> Huber cv. Dorado Gold	
<i>H. stricta</i> Huber cv. Dorado Gold	Marouelli, L.P. 20 (UB)
<i>H. stricta</i> Huber cv. Olivera's Sharonii	Marouelli, L.P. 18 (UB)
<i>H. stricta</i> Huber cv. Iris Red	Marouelli, L.P. 23 (UB)
<i>H. stricta</i> Huber cv. Las Cruces	Marouelli, L.P. 32 (UB)
<i>H. stricta</i> Huber cv. Bucky	Marouelli, L.P. 45 (UB)
<i>H. wagneriana</i> Petersen	
<i>H. latispatha</i> Benth. cv. Orange Gyro	
<i>H. latispatha</i> Benth. cv. Orange Gyro	Marouelli, L.P. 39 (UB)
<i>H. latispatha</i> Benth.	
<i>H. latispatha</i> Benth.	Marouelli, L.P. 40 (UB)
<i>H. spathocircinata</i> Aristeg.	
<i>H. spathocircinata</i> Aristeg.	
<i>H. farinosa</i> Raddi	
<i>H. rivularis</i> Emygdio & Santos	
<i>H. rivularis</i> Emygdio & Santos	Marouelli, L.P. 9 (UB)
<i>H. sampaoiana</i> L. Emygdio	
<i>H. velloziana</i> L. Emygdio	
<i>H. velloziana</i> L. Emygdio	Marouelli, L.P. 16 (UB)
Subgenus <i>Stenochlamys</i> Baker	
<i>H. acuminata</i> L. C. Richard	
<i>H. angusta</i> Vellozo	
<i>H. angusta</i> Vellozo cv. Holiday	Marouelli, L.P. 28 (UB)
<i>H. angusta</i> Vellozo cv. Yellow Christmas	Marouelli, L.P. 29 (UB)
<i>H. angusta</i> Vellozo cv. Orange Christmas	Marouelli, L.P. 30 (UB)
<i>H. laneana</i> Barreiros var. Flava	
<i>H. laneana</i> Barreiros	
<i>H. lacletteana</i> L. Em. et. Em. Santos	
<i>H. lingulata</i> Ruiz & Pavón	Marouelli, L.P. 42 (UB)
<i>H. lingulata</i> Ruiz & Pavón	
<i>H. lingulata</i> Ruiz & Pavón cv. Fan	
<i>H. pseudoaemygdiana</i> L. Emygdio & E. Santos	Marouelli, L.P. 36 (UB)
<i>H. psittacorum</i> L. f. cv. Sassy	
<i>H. psittacorum</i> L. f. cv. Sassy	Marouelli, L.P. 12 (UB)

Continued on next page

Table 1. Continued.

Taxons	Voucher specimens
<i>H. psittacorum</i> L. f. cv. Choconiana	
<i>H. psittacorum</i> L. f. cv. St. Vincent Red	Marouelli, L.P. 14 (UB)
<i>H. psittacorum</i> L. f. cv. St. Vincent Red	
<i>H. psittacorum</i> L. f. cv. St. Vincent Red	
<i>H. psittacorum</i> L. f. cv. Flamingo	Marouelli, L.P. 46 (UB)
<i>H. richardiana</i> Miquel	
<i>H. densiflora</i> cv. Fire flash	Marouelli, L.P. 13 (UB)
<i>H. metallica</i> Planchon & Linden ex Hooker	
<i>H. metallica</i> Planchon & Linden ex Hooker	Marouelli, L.P. 19 (UB)
<i>H. subulata</i> Andersson	Marouelli, L.P. 24 (UB)
<i>H. mathiasiae</i> Daniels & Stiles	
<i>H. hirsuta</i> L. f.	
<i>H. hirsuta</i> L. f.	Marouelli, L.P. 41 (UB)
<i>H. hirsuta</i> L. f. cv. Burle Marx	
<i>H. hirsuta</i> L. f. cv. Yellow Panama	
<i>H. hirsuta</i> L. f. cv. Darrell	
<i>H. longiflora</i> R.R. Smith	
Subgenus <i>Griggsia</i> L. Andersson.	
<i>H. magnifica</i> Kress	Marouelli, L.P. 4 (UB)
<i>H. pogonantha</i> Cufodontes	
<i>H. pogonantha</i> Cufodontes	Marouelli, L.P. 21 (UB)
<i>H. vellerigera</i> Poepping	
<i>H. vellerigera</i> Poepping	Marouelli, L.P. 5 (UB)
<i>H. mariae</i> J.D. Hooker	
<i>H. chartacea</i> Lane ex Barreiros	
<i>H. chartacea</i> Lane ex Barreiros cv. Sexy Pink	
<i>H. chartacea</i> Lane ex Barreiros cv. Sexy Pink	Marouelli, L.P. 17 (UB)
<i>H. chartacea</i> Lane ex Barreiros cv. Sexy Scarlet	
<i>H. chartacea</i> Lane ex Barreiros cv. Sexy Scarlet	Marouelli, L.P. 6 (UB)
<i>H. chartacea</i> Lane ex Barreiros cv. Sexy Orange	
<i>H. chartacea</i> Lane ex Barreiros cv. Amazonita	
<i>H. collinsiana</i> Griggs	
<i>H. collinsiana</i> Griggs	
<i>H. collinsiana</i> Griggs	Marouelli, L.P. 11 (UB)
<i>H. pendula</i> Wawra	
<i>H. pendula</i> Wawra	Marouelli, L.P. 8 (UB)
<i>H. platystachys</i> Baker	
<i>H. juruana</i> Loes	
<i>H. marginata</i> (Griggs) Pittier	
<i>H. marginata</i> (Griggs) Pittier cv. Nutea	
<i>H. rauliniana</i> Barreiros	
<i>H. rauliniana</i> Barreiros	Marouelli, L.P. 38 (UB)
<i>H. rostrata</i> Ruiz & Pavón	
<i>H. rostrata</i> Ruiz & Pavón	
<i>H. rostrata</i> Ruiz & Pavón	
<i>H. rostrata</i> Ruiz & Pavón	Marouelli, L.P. 43 (UB)
<i>H. standleyi</i> Macbride	
<i>H. nariniensis</i> Abalo & G. L. Morales	
Not included in revisions of the genus	
<i>H. santaremensis</i>	
Hybrids	
<i>H. episcopalis</i> Vell. x <i>H. spathocircinata</i> Aristeg. cv. Mantenensis	
<i>H. caribea</i> Lamarck x <i>H. bihai</i> (L.) L. cv. Jacquinii	
<i>H. caribea</i> Lamarck x <i>H. bihai</i> (L.) L. cv. Jacquinii	Marouelli, L.P. 2 (UB)
<i>H. caribea</i> Lamarck x <i>H. bihai</i> (L.) L. cv. Richmond Red	
<i>H. caribea</i> Lamarck x <i>H. bihai</i> (L.) L. cv. Richmond Red	Marouelli, L.P. 34 (UB)
<i>H. psittacorum</i> L. f. x <i>H. spathocircinata</i> Aristeg. cv. Red Opal	
<i>H. psittacorum</i> L. f. x <i>H. spathocircinata</i> Aristeg. cv. Fire Opal	
<i>H. psittacorum</i> L. f. x <i>H. spathocircinata</i> Aristeg. cv. Alan Carle	
<i>H. psittacorum</i> L. f. x <i>H. spathocircinata</i> Aristeg. cv. Golden Torch	
<i>H. psittacorum</i> L. f. x <i>H. spathocircinata</i> Aristeg. cv. Golden Torch	Marouelli, L.P. 10 (UB)
<i>H. x Nickeriensis</i> Maas & deRooy (<i>H. marginata</i> x <i>H. psittacorum</i>)	Marouelli, L.P. 22 (UB)
<i>H. x episcopalis</i> Vell.	

Table 2. Outgroup accessions representing five families of the order Zingiberales.

Species	Family
<i>Musa coccinea</i> Andr.	Musaceae
<i>Costus barbatus</i> Susseng.	Costaceae
<i>Alpinia purpurata</i> (Vieill.) Schum.	Zingiberaceae
<i>Ischnosiphon ovatus</i> Koern.	Marantaceae
<i>Phenakospermum guyanense</i> (L. C. Rich.) Endl. Ex Miq.	Strelitziaceae

Each polymerase chain reaction (PCR) mixture was composed of the following components: 0.2 µg/mL bovine serum albumin; 0.2 mM each dNTP; 0.4 µM primer; 1 U *Taq* polymerase; 7.5 ng DNA; 3.42 µL ultra-pure sterile water, and 1X *Taq* DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂). PCR-cycling conditions were 40 cycles of DNA denaturation at 92°C for 1 min, primer annealing at 35°C for 1 min and extension at 72°C for 2 min, with a final incubation at 72°C for 7 min following cycling. The PCR products were visualized after electrophoresis on 1.5% agarose gels containing ethidium bromide and including 1-kb DNA ladders. The DNA patterns were scored as 1 for presence and 0 for absence of a band, yielding a binary matrix.

For phenetic analysis, the data were used to construct a genetic similarity matrix employing the Jaccard coefficient. A dendrogram was constructed using UPGMA (unweighted pair group method with arithmetic mean), implemented in NTSYS-PC, version 2.02 (Rohlf, 1993) and the cophenetic correlation coefficient calculated using the Mantel test. Non-parametric bootstrap analysis with 1000 random samplings, using BOOD v. 3.0 (Coelho, 2001), was done to assess group support, where bootstrap values above 95% were considered to be highly significant, values between 94-70% were considered to be moderate and values between 69-51% were considered to be weakly supported. However, if the moderate or weak values are repeated in many analyses with different markers, they may indicate support for the group (Hillis and Bull, 1993; Li, 1997).

The cladistic analysis was performed under the maximum parsimony criterion using PAUP 4.0b10 (Swofford, 2002), considering each character as equally weighted and unordered. Heuristic searches were performed using random taxon addition, tree bisection-reconnection branch swapping and ACCTRAN character optimization. Bootstrap analysis was used to assess the degree of support for each branch, and tree statistics such as consistency index (Kluge and Farris, 1969) and retention index (Farris, 1989) were computed.

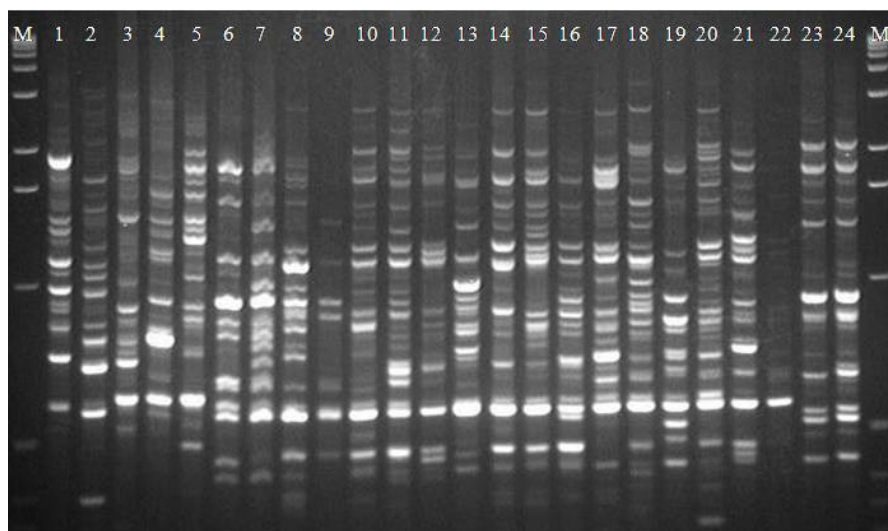
RESULTS AND DISCUSSION

Fourteen primers were selected (OPA-13, OPA-20, OPB-5, OPB-11, OPC-2, OPD-7, OPE-9, OPF-5, OPG-9, OPO-14, OPP-3, OPV-16, OPV-18, and OPX-18) from the screening of 150 primers. The selected primers produced a high degree of polymorphism, where of a total of 374 amplified bands, 231 were polymorphic (Table 3), with each primer giving a mean of 16.5 polymorphic bands. This high polymorphism may be related to the diversity of species and families included in this study (Figure 1).

In the phenetic analysis, the cophenetic correlation coefficient (Mantel test) revealed a good fit between the calculated distances graphically presented and the similarity matrix, with a value of $r = 0.89$ for the dendrogram, where according to Sokal and Rohlf (1962), values of cophenetic correlation above 0.80 are preferable. The combination of the 231

Table 3. Primer names, primer sequence and number of polymorphic bands produced by the 14 primers selected for the RAPD study of *Heliconia* accessions.

Primer	Sequence (5'-3')	Polymorphic bands
OPA-13	CAG CAC CCA C	15
OPA-20	GTT GCG ATC C	24
OPB-5	TGC GCC CTT C	18
OPB-11	GTA GAC CCG T	20
OPC-2	GTG AGG CGT C	20
OPD-7	TTG GCA CGG G	13
OPE-9	CTT CAC CCG A	20
OPF-5	CCG AAT TCC C	12
OPG-9	CTG ACG TCA C	15
OPO-14	AGC ATG GCT C	13
OPP-3	CTG ATA CGC C	6
OPV-16	ACA CCC CAC A	14
OPV-18	TGG TGG CGT T	23
OPX-18	TGG CAA GGC A	18

**Figure 1.** PCR banding pattern of *Heliconia* accessions and outgroup species revealed by RAPD markers on 1% agarose gels: amplification using primer OPA-20. M = 1-kb ladder (Invitrogen). Lanes 1-5 = outgroup species and lanes 6-24 = *Heliconia* accessions.

polymorphic markers strongly supported the monophyly of *Heliconia*, with a bootstrap value of 100% (Figure 2), in relation to the five outgroup accessions included in the analysis.

The heliconias were divided into a large group, consisting of more than 90% of all accessions, subdivided into two (Clade 2 and Clade 3), and a related group, containing *H. latipatha* accessions (Clade 1 - bootstrap 99%). Clade 2 was subdivided into four subgroups. The first subclade included *H. psittacorum*, *H. densiflora*, *H. richardiana*, and *H. collinsiana* accessions; a larger sister subclade comprised *H. rostrata*, *H. juruana*, *H. standley*, *H. platystachys*, *H. marginata*, and *H. rauliniana* accessions. A second subclade comprised *H. magnifica*, *H. pogonantha*, *H. marginata*, *H. vellerigera* accessions, and cultivars of *H. chartacea* (bootstrap 91%). The third subclade included *H. metallica*, *H. longiflora*, *H. mathiasiae*, and cultivars of *H. hirsuta* (bootstrap 51%).

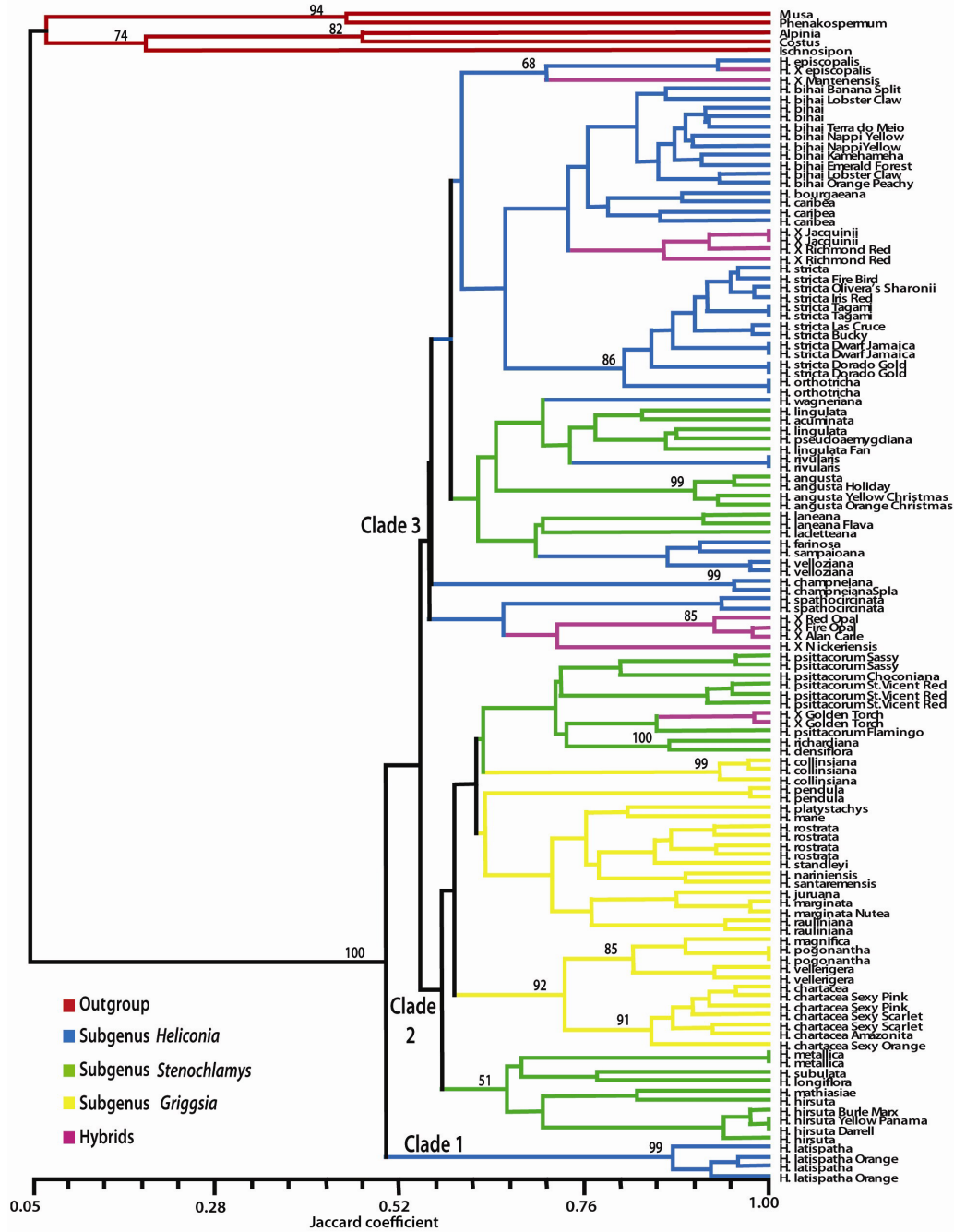


Figure 2. UPGMA dendrogram of the 124 accessions studied obtained by genetic similarity analysis using the Jaccard coefficient, generated by NTSYS-PC, with 231 RAPD markers. Bootstrap values (>50%) are indicated above branches. The cophenetic correlation coefficient is 0.89.

Clade 3 possessed a more complex structure, where it was possible to identify four subclades. The first subclade was composed of a small grouping of *H. episcopalis* and its probable hybrids (*H. x mantenensis* and *H. x episcopalis*) (bootstrap 68%), a subgroup composed of *H. bihai*, *H. bourgaeana*, *H. caribea* accessions, and the hybrids of *H. bihai* x *H. caribea* (ex., *H. bihai* x *H. caribea* cv. Richmond Red), and a subgroup composed of cultivars of *H. stricta* and of *H. orthotricha* accessions (bootstrap 86%). The second subclade comprised a grouping composed of *H. lingulata*, *H. acuminata*, *H. pseudoaemygdiana*, *H. rivulares*, *H. angusta* accessions (bootstrap 99%), *H. laneana*, *H. lacletteana*, *H. farinosa*, *H. sampaioana*, and *H. velloziana*. The third and fourth sister subclades were composed of two *H. champneiana* accessions and a grouping comprising *H. spathocircinata* accessions and the hybrids *H. psittacorum* x *H. spathocircinata* cultivars Red Opal, Fire Opal and Alan Carle.

Of the conventional *Heliconia* subgenera, the subgenus *Griggsia* was monophyletic, with all assigned accessions being restricted to Clade 2. In addition, the subgenus *Heliconia* was, with the exception of *H. latispatha*, restricted to Clade 3. The subgenus *Stenochlamys*, however, was clearly polyphyletic in the analysis.

The maximum parsimony analysis of the data resulted in 2357 trees, from which was generated a strict consensus tree with 616 steps (Figure 3). All 231 polymorphic RAPD characters were considered to be informative for parsimony. This analysis showed a low consistency index (0.375), high homoplasy index (0.625), and generally low bootstrap support for the deeper branches. Nevertheless, it was possible to identify the Heliconiaceae family as a monophyletic group as suggested by previous studies (Kress et al., 2001; APG II, 2003; Kress and Specht, 2006). As in the phenetic analysis, the subgenus *Griggsia* was monophyletic. The subgenus *Heliconia* was also largely monophyletic, where, unlike in the phenetic analysis, *H. latispatha* grouped with other subgenus *Griggsia* accessions. However, an exception in the cladistic analysis was that *H. farinosa*, *H. sampaioana*, and *H. velloziana* grouped distantly from other *Heliconia* accessions. The species currently assigned to the subgenus *Stenochlamys*, *H. psittacorum*, *H. densiflora*, and *H. lingulata* were clearly allied to the other subgenus *Griggsia* species in our analysis. *H. psittacorum*, *H. densiflora* and *H. richardiana* are plants with erect inflorescences classified in the *Stenochlamys* subgenus, but are clearly closely related to the subgenus *Griggsia*, which includes species with generally pendant inflorescences (Andersson, 1985, 1992). This growth form is therefore phylogenetically misleading. Kress (1984) and Castro et al. (2007) earlier hypothesized that the section *Griggsia* is not monophyletic, as opposed to Andersson's hypothesis (1992). As in the phenetic analysis, the subgenus *Stenochlamys* was clearly polyphyletic.

In the phenetic and maximum parsimony analysis with RAPD markers, large groups composed of *H. bihai*, *H. stricta*, *H. psittacorum*, *H. chartacea*, *H. angusta*, and *H. hirsuta* cultivars and hybrids were observed, showing that in spite of these species having a large variety of forms (Berry and Kress, 1991), the cultivars show a high genetic similarity (Figures 2 and 3). The results also indicate that RAPD markers are extremely useful for the identification and assignment of unknown *Heliconia* cultivars to their species of origin. In this respect, the group composed of *H. episcopalis* and its probable hybrids (*H. x mantenensis* and *H. x episcopalis*), by *H. bihai*, *H. bourgaeana*, and *H. caribea* accessions, and the hybrids of *H. bihai* x *H. caribea*, by *H. stricta* cultivars and accessions of *H. orthotricha*, may be compared to the classification by Andersson (1992), which included these species in the subgenus *Heliconia*. Interestingly, the interspecific hybrids of the related species *H. episcopalis* and *H. bihai* grouped with one of their parentals.

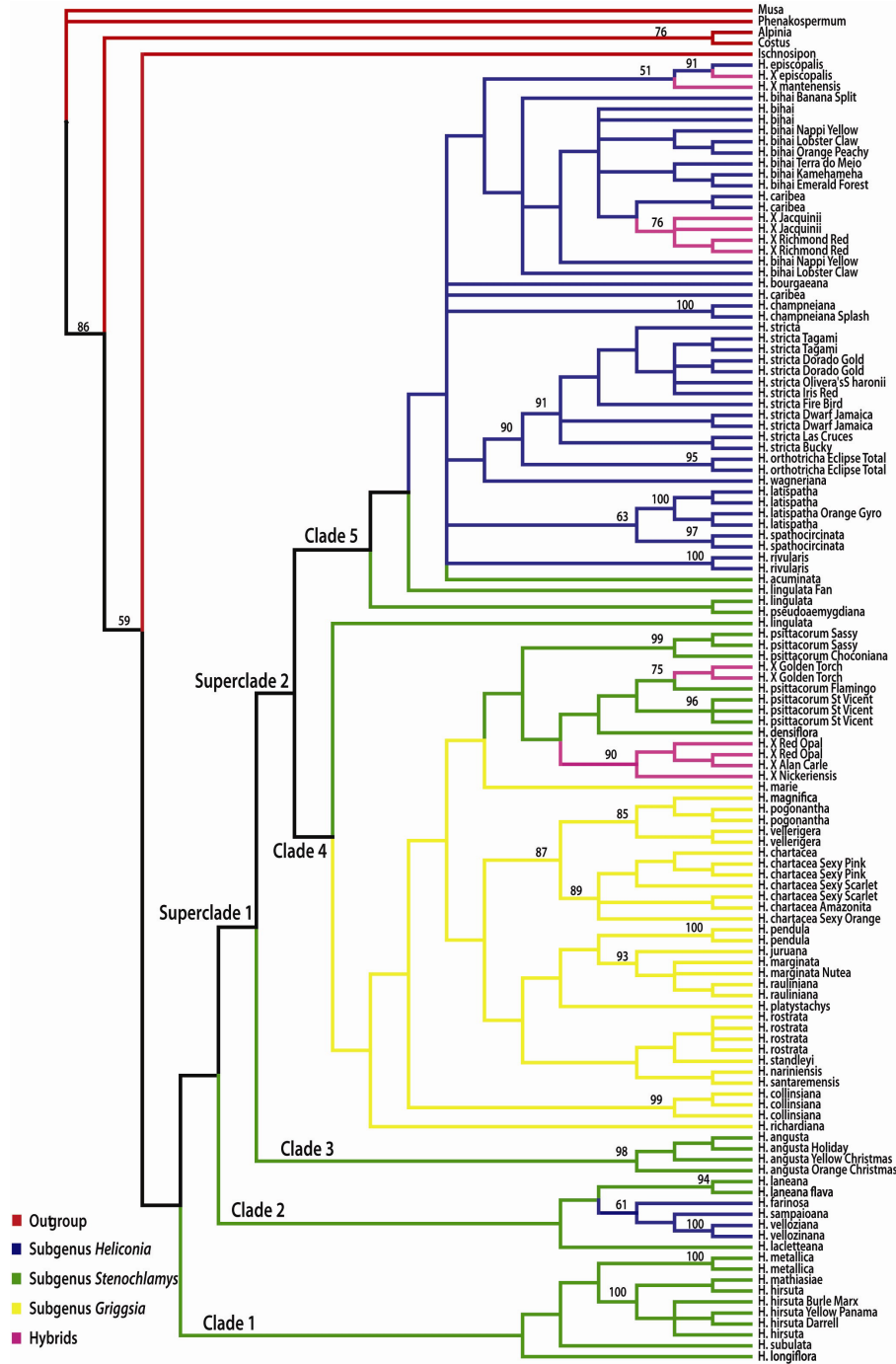


Figure 3. Strict consensus tree of 124 accessions obtained from maximum parsimony analysis calculated by PAUP* v.4, with 231 RAPD markers. Bootstrap values (>50%) are indicated above branches.

The group composed of *H. lingulata*, *H. acuminata*, *H. pseudoaemygdiana* and *H. rivulares*, *H. angusta*, *H. laneana*, *H. lacletteana*, *H. farinosa*, *H. sampaioana* and *H. velloziana* shares species from distinct subgenera. *H. rivulares*, *H. farinosa*, *H. sampaioana*, and *H. velloziana* are endemic to Brazil, and are classified in the section *Farinosae* of the subgenus *Heliconia* (Kress et al., 1993). *H. lingulata*, *H. acuminata*, *H. pseudoaemygdiana*, *H. angusta*, *H. laneana*, and *H. lacletteana* are from the same subgenus, but distinct sections.

H. lingulata and *H. pseudoaemygdiana* are from the section *Lanea*, and *H. acuminata*, *H. angusta*, *H. laneana*, and *H. lacletteana* are from the section *Stenochlamys*.

The subgroup of *H. metallica*, *H. longiflora*, *H. mathiasiae*, and *H. hirsuta* confirms the Andersson (1985, 1992) classification, which includes these species in the subgenus *Stenochlamys*, but in distinct sections.

H. metallica and *H. mathiasiae* are from the section *Cannastrum*, while *H. longiflora* and *H. hirsuta* are from the section *Zingiberastrum* (Andersson, 1985).

In general, both analyses resulted in trees with similar topologies. However, differences in some internal groupings were observed: the group with *H. latispatha* cultivars were outliers in the phenetic analysis, but grouped with accessions of *H. spathocircinata* in the maximum parsimony analysis. The discrepancies between the analyses may have occurred due to the generally low clade support for deeper branches and/or to the fact that the phenetic analysis is based on a distance matrix (the character matrix is transformed into distance matrix), and parsimony (cladistic analysis) is based on character state (the characters are directly analyzed) (Schneider, 2003).

The data obtained with RAPD markers permitted a large genome coverage (Ferreira and Grattapaglia, 1998), and facilitated the identification of groups that corresponded well along species lines, where cultivars and hybrids clustered with their corresponding parents. The low bootstrap support for the more deeply branched clades and the incongruence in the relationships between some groups did not allow us to confidently reject the current division of *Heliconia* into its currently accepted subgenus and section assignments, as proposed by Andersson (1985, 1992), Kress et al. (1993) and Kress (1997). However, our data do give reason to doubt the current taxonomic structure of the genus, particularly in the position of the polyphyletic subgenus *Stenochlamys*. To extend these studies, DNA sequence-based analyses are required to further clarify the phylogenetic structure of *Heliconia*.

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