BIODIVERSITAS
Volume 20, Number 5, May 2019
Pages: 1266-1283

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200505

Species diversity and phylogenetic analysis of *Heliconia* spp. collections of Purwodadi Botanic Garden (East Java, Indonesia) inferred by *rbc*L gene sequences

LIA HAPSARI[1,♥], TRIMANTO[1,♥♥], DIDIK WAHYUDI[2]

[1]Purwodadi Botanic Garden, Research Center for Plant Conservation and Botanic Gardens, Indonesian Institute of Sciences. Jl. Raya Surabaya-Malang Km 65, Purwodadi, Pasuruan 67163, East Java, Indonesia. Tel./fax.: +62-343-615033, ♥email: hapsari.lia@gmail.com, lia.hapsari@lipi.go.id, ♥♥triman.bios08@gmail.com; trim006@lipi.go.id

[2]Departement of Biology, Universitas Islam Negeri Maulana Malik Ibrahim. Jl. Gajayana No.50, Malang 65144, East Java, Indonesia

Manuscript received: 27 February 2019. Revision accepted: 9 April 2019.

Abstract. *Hapsari L, Trimanto, Wahyudi D. 2019. Species diversity and phylogenetic analysis of* Heliconia *spp. collections of Purwodadi Botanic Garden (East Java, Indonesia) inferred by* rbc*L gene sequences. Biodiversitas 20: 1266-1283. Heliconia* L. is a single genus in the family Heliconiaceae, with approximate consists of 200 species. It has wide morphological variations among and within species which led to problems in species identification. Species diversity and phylogenetic analysis using morphology and *rbc*L marker subjected to 17 *Heliconia* spp. living collections of Purwodadi Botanic Garden (PBG) have been conducted. The *rbc*L gene located in chloroplast genome is one of appropriate proposed marker for plant barcoding assessment. This study aimed to study morphology and genetic variability of the PBG Heliconiaceae collections, to confirms the species name for a more accurate identity record and to reveal the diversity and phylogenetics of the species. Morphological characterization showed high variability among *Heliconia* species, which included 3 subgenera (*Heliconia*, *Stenochlamys*, and *Griggsia*) and 1 hybrid. Each species possessed unique morphological characteristics. The common morphological characters which distinguished among and within *Heliconia* species includes leaf form, inflorescence type, and bract characteristics. Key to the *Heliconia* species examined is presented in this paper. However, molecular confirmation using *rbc*L sequences showed high conservation level (0.932) and low genetic variability. About 656 nucleotides were monomorphic and 33 positions were polymorphic which comprised 18 singleton variable sites and 15 parsimony informative sites. Twelve haplotypes were produced with haplotype diversity value 0.8952. Pairwise distance analysis shows that they were shared high similarity of *rbc*L sequences with very low genetic distance (0.022 to 0.000). The topology of phylogenetic tree resulted by Neighbour-Joining algorithm has the best grouping and be able to explain the relationship among species of *Heliconia*, although supported by low bootstrap (65). It was separated into two clades following its subgen. classification. Clade 1 consists of subgen. *Heliconia* and *Griggsia*; while clade 2 consists of subgen. *Heliconia* and *Stenochlamys*; also hybrid species. Further, separation of deeper branchings (section) was inconsistent and unclear. Upon this study, *rbc*L marker was considered too conserved thus less valuable for phylogenetic analysis at lower taxa among and within *Heliconia* spp. However, *rbc*L was suggested to distinguish at higher level taxa between closely related genus and above.

Keywords: *Heliconia*, identification, molecular marker, morphology, phylogenetic, *rbc*L

INTRODUCTION

Heliconia L. is a tropical perennial herbaceous plant with brilliant and colorful flowering bracts. The genus *Heliconia*, formerly included in the family Musaceae, and has been variously associated with the Strelitziaceae, but is now the only genus under Heliconiaceae of the order Zingiberales (Kress 1990a). It is popularly known as *pisang hias* in Indonesia, due to its morphological appearance which is similar to *pisang* or banana, and utilized mostly for *hiasan* or ornamentals purpose. It is also known as "false bird of paradise plant", because of its similarity with *Strelitzia* (Maria et al. 2014).

There are approximately 200 species of *Heliconia* spp., occurring throughout the American tropics and Melanesia (Andersson 1998). The center of diversity of the genus is found along the northern Andes (Colombia and Ecuador) extending into southern Central America (Panama and Costa Rica; Andersson 1989). Then it is distributed to the Pacific Ocean islands westward to Indonesia, and neotropics (Kress 1990a; Berry and Kress 1991). Preliminary classification of *Heliconia* proposed by Kress (1984, 1990a), Kress and Beach (1994) and Andersson (1981, 1985, 1992) is comprised of five subgenera, i.e. *Heliconia*, *Taeniostrobus* (Kuntze) Griggs, *Heliconiopsis* (Miq.) W.J. Kress, *Stenochlamys* Baker, and *Griggsia* L. Anderss. Further, each subgen. is classified to some sections (in total 23 sections) which distinguished primarily by morphological characteristics.

Heliconia plant is traditionally propagated mostly through rhizomes and seeds (limited). Most species inhabit moist or wet regions, but some are found in seasonally dry areas, which may grow in open and secondary sites such as along roadsides, on river banks, and in forest light gaps; and some species readily invade and colonize the newly opened areas (Kress 1990a; Kress 1990b; Krauss et al. 2008; Booth 2010; Isaza et al. 2012). Nowadays, *Heliconia* spp. are widely cultivated as ornamental garden plants in

the tropics, and have become increasingly popular as indoor decorative cut-flowers for bouquets and arrangements (Marouelli et al. 2010; Maria et al. 2014).

The genus *Heliconia* has classified as a group of highly variable and diverse plants. The prominent characters of *Heliconia*, includes large leaves with short to long petioles and blades possessing transverse venation; large, usually colorful, bracteate inflorescences, and arillate seeds. Species and cultivar identification of *Heliconia* are primarily based on morphological difference and coloration of the inflorescences (Kress 1990a; Berry and Kress 1991). Further, wide variations exist among and within species. The broad diversity of *Heliconia* species, varieties, hybrids, and cultivars has caused confusion and uncertainty regarding the correct denomination of the species triggering problems at technical/scientific levels also commercial (Kumar et al. 1998; Sultana and Hasan 2008; Isaza et al. 2012). In addition, there are taxonomic confusions and uncertainties about the number of species and the relationships among them (Marouelli et al. 2010). Therefore, molecular studies using DNA based methods such as Random Amplified DNA/RAPD (Kumar et al. 1998), Amplified Fragment Length Polymorphism/AFLP (Isaza et al. 2012), Inter-Simple Sequence Repeats/ISSR (Pereira et al. 2015), DNA barcode markers from chloroplast and/or nucleus genome (CBOL 2009), etc. are necessary to conduct for a better understanding of the species boundaries of this family.

Purwodadi Botanic Garden - Indonesian Institute of Sciences (PBG) is located in Pasuruan, East Java, Indonesia. The first *Heliconia* collection of PBG was recorded in 1976, located at Vak XI.D along with Musaceae collection (Hapsari 2011). In the 80's, due to field re-arrangements of the collections, it was moved to Vak V.D.II until now. The *Heliconia* specimens were collected through explorations, exchanges, and donations from personals and other botanic gardens (Lestarini et al. 2012). Last field inspection in 2017 by Registration Unit, the total *Heliconia* collection was about 17 coll. numbers (Fauziah, 2017). Identification both morphology and molecular are required subjected to *Heliconia* spp. collections of PBG for a more accurate identity record and further research and development purposes.

DNA barcoding is a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed-upon position in the genome. The *rbc*L exon size approximately at full length 1,400 bp provides many characters that can be utilized in phylogenetic analysis (Smith et al. 1993; Newmaster et al. 2006). Further, it has high conservation level and evolves at slow rate which valuable for genetic diversity and phylogenetic studies (Chase et al. 1993; Clegg 1993; Hollingsworth et al. 2009). The *rbc*L gene located in chloroplast genome encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) which involved in the first major step of carbon dioxide fixation (Chase et al. 1993). It was also allegedly linked to the evolutionary process of adaptation to the environment and climate change (Hasegawa et al. 2009). Although *rbc*L gene variability in many plant species was considered too conserved, previous molecular studies reported *rbc*L marker were often used and suitable for Monocots, particularly Zingiberales order, including Heliconiaceae (Smith et al. 1993; Kress 1995; Kress et al. 2001; Kress et al. 2002; Davis et al. 2004; Kress and Specht 2006; Newmaster et al. 2006; Kress and Erickson, 2007; Handique et al. 2013).

The aim of this study was to reveal the species diversity of the *Heliconia* spp. collections of PBG based on morphology and to confirm the species identity using molecular *rbc*L barcode, also to elucidate the phylogenetic relationship among and within species. More accurate identification of the plant collection is very important for botanic garden records. Further, appropriate identification and characterization of plant materials are essential for the successful conservation of plant resources and to ensure their sustainable uses (Leadlay and Jury 2006; Ibrahim et al. 2010). This study also aimed to fill in the gaps of bio-informatics data of *Heliconia* spp. from Indonesia. Storage of genetic information through a centralized GenBank DNA database will allow biodiversity data to be preserved and provide intellectual property protection and establish commercial benefits to owners of biological resources (Hapsari et al. 2018).

MATERIALS AND METHODS

Plant materials

In total, 17 living plant specimens of *Heliconia* spp. collections of PBG located in Pasuruan, East Java, Indonesia have been studied. It was collected from wide areas from Tropical America, Malagasy, to Indonesia i.e. Java and Mollucas. In addition, their close relative species from the Zingiberales Order were used as outgroups. It comprised two species of Strelitziaceae i.e. *Ravenala madagascariensis* (pisang kipas) and *Phenakospermum guyannense*, also two species of Musaceae i.e. wild seeded bananas *Musa balbisiana* (pisang klutuk ijo) and *Musa acuminata* var. *flava* (pisang jantung kuning) (Table 1).

Procedures

Morphological characterization

Morphological characterization was conducted to the living plant specimens using modified descriptors for *Heliconia* by Guimarães et al. (2014), both qualitative and quantitative characteristics on vegetative and generative parts of the plant. Vegetative parts observed include pseudostem, leaf form, leaf blade, petiole, and ligule. Generative parts observed includes inflorescence, peduncle, rachis, bract, perianth, pedicel and fruit.

Molecular procedure

Molecular analysis was conducted at Plant Physiology Laboratory of Biology Department, University of Brawijaya, Malang, Indonesia. The fresh young leaf sample was taken for molecular analysis, one individual per *Heliconia* coll. number. Total genomic DNAs were extracted using Promega Wizard® Genomic DNA Purification Kit (Madison, WI, USA) followed the manufacturer's protocols for plant.

Table 1. Plant materials examined of ingroup (Heliconiaceae) and outgroup (Strelitziaceae and Musaceae) collections of Purwodadi Botanic Garden, East Java, Indonesia

Code	Coll. number	Registration number	Species name	Subgen.*	Section*	Locality**	Genbank acc. number
H1	V.D.II.4	Not available	*Heliconia wagneriana* Petersen	*Heliconia*	*Heliconia*	East Java	MK238287
H2	V.D.II.28	P1997060328	*Heliconia hirsuta* L.f.	*Stenochlamys* Baker	*Zingiberastrum*	Seram Island, Mollucas	MK238288
H3	V.D.II.19	P199507132	*Heliconia latispatha* Benth.	*Heliconia*	*Tortex* L. Anderss.	Morotai Island, Mollucas	MK238289
H4	V.D.II.6	Not available	*Heliconia wagneriana* Petersen	*Heliconia*	*Heliconia*	East Java	MK238290
H5	V.D.II.8	P19780419	*Heliconia bihai* (L.) L.	*Heliconia*	*Heliconia*	West Java	MK238291
H6	V.D.II.20	P19940414	*Heliconia latispatha* Benth.	*Heliconia*	*Tortex* L. Anderss.	West Java	MK238292
H7	V.D.II.2	Not available	*Heliconia psittacorum* L.f.	*Stenochlamys* Baker	*Stenochlamys* (Baker) Schum.	Malagasy	MK238293
H8	V.D.II.17	P19940411	*Heliconia psittacorum x H. spathocircinata* Aristeg. cv. Golden Torch	Hybrid	-	West Java	MK238294
H9	V.D.II.15	P1994047	*Heliconia latispatha* Benth.	*Heliconia*	*Tortex* L. Anderss.	West Java	MK238295
H10	V.D.II.16	P1994049	*Heliconia psittacorum x H. spathocircinata* Aristeg. cv. Golden Torch	Hybrid	-	West Java	MK238296
H11	V.D.II.12	Not available	*Heliconia collinsiana* Griggs	*Griggsia* L. Anderss.	*Pendulae* (Griggs) W.J. Kress, ined.	Guatemala	MK238297
H12	V.D.II.11	Not available	*Heliconia rostrata* Ruiz & Pav.	*Griggsia* L. Anderss.	*Rostratae* W.J. Kress, ined.	Trop. America	MK238298
H13	V.D.II.25	P19950616	*Heliconia latispatha* Benth.	*Heliconia*	*Tortex* L. Anderss.	Trop. America	MK238299
H14	V.D.II.7	P19780418	*Heliconia metallica* Planch. & Linden ex Hook.	*Stenochlamys* Baker	*Cannastrum* L. Anderss.	Brazil	MK238300
H15	V.D.II.26	P19950615	*Heliconia chartacea* Lane ex Barreiros	*Griggsia* L. Anderss.	*Pendulae* (Griggs) W.J. Kress, ined.	Brazil	MK238301
H16	V.D.II.24	P19941239	*Heliconia latispatha* Benth.	*Heliconia*	*Tortex* L. Anderss.	Trop. America	MK238302
H17	V.D.II.14	P1994046	*Heliconia latispatha* Benth.	*Heliconia*	*Tortex* L. Anderss.	West Java	MK238303
S1	V.D.II.1	P19810641	*Ravenala madagascariensis* Sonn.	-	-	Malagasy	MK238283
S2	V.D.II.13	P1954126	*Phenakospermum guyannense* (A.Rich.) Endl. ex Miq.	-	-	Brazil	MK238284
M1	XXIV.D.1	P1980041	*Musa balbisiana* Colla	-	*Eumusa*	Pasuruan, East Java	MK238285
M2	XXIV.A.29	P19810982	*Musa acuminata* var. *flava* (Ridl.) Nasution	-	*Eumusa*	Tuban, East Java	MK238286

Notes: S = Strelitziaceae, M = Musaceae, H = Heliconiaceae. *Subgen. references = Kress (1984, 1990a), Kress and Beach (1994), Andersson (1981, 1985, 1992). **Locality reference = Lestarini et al. (2012)

Amplification of the *rbc*L marker was accomplished using primer pairs of *rbc*L-1F (5'- ATG TCA CCA CAA ACA GAA AC-3') and *rbc*L724R (5'- TCG CAT GTA CCT GCA GTA GC-3') referred to CBOL (2009). PCR reactions were conducted in a 30 µL volume contains of 15 µL of DreamTaq Green PCR Master Mix (2x) from Thermo Scientific, California, USA (Taq DNA polymerase, 2x DreamTaq Green buffer, 0.4 mM each of dNTPs and 4 mM $MgCl_2$), 3µL of 5 pmol each of forward and reverse primers, and 3µL of nuclease-free water. PCR thermal cycling program used for *rbc*L amplification consists of initial denaturation temperature at 95 °C for 5 minutes; followed by 30 cycles of denaturation for 45 seconds at 95 °C, annealing for 45 seconds at 60.8 ºC, and extension for 45 seconds at 72 ºC. Final extension carried out for 10 minutes at 72 ºC. Amplified products were then purified and sequenced at 1st *BASE Laboratories* Sdn Bhd, Malaysia using ABI PRISM 3730xl Genetic Analyzer developed by Applied Biosystems, USA.

Data analysis

Morphological data analysis

Morphological characteristics data of each *Heliconia* species were analyzed descriptively. The species identification was referring to identification guides of *Heliconia* books (Berry and Kress 1991; Arnold 2013; Hintze 2014); protologues of some type species of *Heliconia* (Linnaeus 1772, 1781; Bentham 1846; Planchon and Linden 1862; Scumann 1900; Griggs 1903, 1915; Hodge 1941; Kress 1981, 1983, 1984, 1990a, 1990b; Barreiros 1972), Flora Peruviana (Ruiz Lopez and Pavon, 1802), Flora de Brasilia (Petersen 1890); Flora of Guatemala (Standley and Steyemark 1952); Flora of Java (Backer and Bakhuisen van den Brink 1968), Flora Mesoamericana (Kress 2008), scientific journals, and *Heliconia* online databases incudes *Heliconia* Society Puerto Rico (http://www.heliconiasocietypr.org/), Plants of the world online of Kew Science (http://www.plantsoftheworldonline.org), Smithsonian Tropical Research Institute (https://biogeodb.stri.si.edu/), Centre for Agriculture and Bioscience International (https://www.cabi.org/), Fairchild Botanic Garden/ FTBG (https://www.fairchildgarden.org/), iNaturalist.org (https://www.gbif.org), etc. Further, the distinguishing characters among species and subgenus were studied through synapomorphy, autapomorphy and apomorphy characters analysis.

Molecular data analysis

DNA sequences of *rbc*L were evaluated using ABI sequences Scanner v.10. Multiple sequences alignments were performed using ClustalW program followed by visual adjustment (MEGA6.06 software). Genetic diversity including nucleotide mutations was analyzed with DnaSP ver. 5.10.01. Median Joining analysis was employed using Haplotype Network 5.0.0.3 to analyze the genetic diversity and reconstruct haplotype distribution map. Phylogenetic reconstructions were performed using MEGA6.06 based on evolution model of Kimura 2 parameter (K2P) using Neighbor-Joining (NJ), Maximum Parsimony (MP), and Maximum Likelihood (ML) algorithms with 1000 bootstrap replications; pairwise distance analysis also performed to generate genetic distances (Seltman et al. 2003; Tamura et al. 2013). Bootstrap support was categorized as strong (>85 %), moderate (70-85 %), low (50-69 %), and very low (<50 %) (Kress et al. 2002).

RESULTS AND DISCUSSION

Based on morphological characterization to 17 *Heliconia* living collections of PBG showed that 15 coll. numbers were matched to 9 *Heliconia* species, includes *H. bihai*, *H. chartacea*, *H. collinsiana*, *H. hirsuta*, *H. latispatha*, *H. metallica*, *H. psittacorum*, *H. rostrata*, and *H. wagneriana*. Two coll. numbers were considered as hybrid i.e. *H. psittacorum* x *spathocircinata* cv. Golden Torch (Table 1). Fifteen *Heliconia* spp. examined (hybrid excluded) in this sudy were included in 3 subgenera i.e. *Heliconia*, *Griggsia*, and *Stenochlamys*. Further, each subgen. also divided into some sections such as *Heliconia*, *Tortex*, *Pendulae*, *Rostratae*, *Stenochlamys*, *Zingiberastrum* and *Cannastrum* (Table 1).

The key morphological characteristics to the *Heliconia* species collections of PBG was presented in this paper. Further, the complete morphological characterization and information of each *Heliconia* species collections of PBG was also described.

***Heliconia* L.**

Heliconia L., Mant. Pl. 2: 147 (1771) (Figure 1.A-B-C-D-E-F-G-H-I-J).

General morphological description. Medium to large-sized, perennial, rhizomatous herbs forming clusters of erect leafy shoots in groups of few (1-2) to many (> 50). *Pseudostem* composed of overlapping sheathing leaf bases. *Leaves* simple, distichous, petiolate, usually large; *petiole* i.e. long-length resembling bananas (musoid), medium-length resembling cannas (cannoid) and shorth-length resembling gingers (zingiberoid); *blades* with transverse venation, the base unequal on either side of the midrib, usually green on both sides. *Inflorescence* terminal, erect or pendent, consisting of brightly colored, leaflike, distichous or spirally arranged bracts, each subtending a cluster of flowers. *Flowers* bisexual, each subtended by a membranous floral bract; *perianth* consisting of 2 whorls united at the base with varying degrees of fusion within and between the whorls; *calyx* with 2 partially fused sepals and 1 nearly free sepal; *corolla* with 3 petals, fused except for free margins opposite the free sepal; *pollen* bearing stamens 5, attached to the base of the perianth tube; staminode 1, of variable size and shape, opposite the free sepal; *ovary* inferior, 3-celled; *ovules* solitary, erect; style 1. *Fruit* 1-3-seeded drupe, mostly blue, some red or orange, at maturity; *seed* surrounded by stony, roughened endocarp.

Key to the *Heliconia* species collections of Purwodadi Botanic Garden

1	a.	Inflorescence erect	2
	b.	Inflorescence pendent	3
2	a.	Leaf blade upper and lower surface green-purplish	*H. metallica*
	b.	Leaf blade upper and lower surface light green to green	4
3	a.	Rachis straight, bract inverted claw-shaped, distichous, very hairy (velvety)	*H. rostrata*
	b.	Rachis slightly flexuosus, bract inverted boat-shaped, spiral, glabrous	5
4	a.	Leaf form zingiberoid-type	*H. hirsuta*
	b.	Leaf form musoid-type	6
5	a.	Bract color pink with yellow to light green at tip and margin, slightly waxy	*H. chartacea*
	b.	Bract color continuous red-orange, very waxy at apex	*H. collinsiana*
6	a.	Bract deeply boat-shaped	7
	b.	Bract shallow and narrow boat-shaped	8
7	a.	Bracts spirally arranged	*H. latispatha*
	b.	Bracts distichously arranged	9
8	a.	Rachis straight, bracts distichously arranged, red to orange color, perianth with distal dark green spot	*H. psittacorum*
	b.	Rachis slightly flexuosus, bracts spirally arranged, golden yellow color, perianth with distal faint green spot	*H. psittacorum* × *H. spathocircinata*
9	a.	Bract color bright red to orange, with green tip and yellow to green margin	*H. bihai*
	b.	Bract color green to pale red, with light green tip and green margin	*H. wagneriana*

***Heliconia* L.**

Subgen. *Heliconia* (Figure 1.A-B-C).

Key characters. *Inflorescence* erect, *bracts* deeply boat-shaped (cymbiform) and *flowers* greenish on untwisted pedicels.

***Heliconia bihai* (L.) L.**

Heliconia bihai (L.) L., Mant. P1. 211. (I771); Griggs, Bull. Torrey Bot. Club 30 (12): 656 (1903); Hodge, Contribut. Gray Herbarium Harvard Univ. 135: 135 (1941); Standley & Steyemark, Fl. Gua. 24 (III): 181 (1952); Backer & Bakhuisen van den Brink III, 206: 40 (1968); Kress, Acta Bot. Bras. 4 (1): 164 (1990) (Figure 1.A)

Vernacular name. Parrots's flower, Macaw flower.

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 58-98 cm, green with large brown blotches and waxy. *Leaf* form musoid-type; leaf blade shape oblong, oblique at base and mucronate at apex, 60-92 cm x 21-29 cm (ratio 2.9-3.2); leaf upper color dark green and lower pale green; midrib upper color green and lower light green with reddish line on central; leaf surface not waxy and dull on both sides, very undulated, not ripped. *Petiole* 45-82 cm, green, glabrous, slightly waxy. *Ligule* color green with large brown blotches. *Inflorescence* erects with short peduncle, 3.0-4.0 cm, bright red-orange, glabrous. *Rachis* straight, 20-21 cm, bright red-orange, glabrous. *Bract* deeply boat-shaped, 7-12, distichous arrangement, middle bracts 10-11 cm x 2.8-3.0 cm, appearance very compact (distance between bracts 0-0.5 cm); color at base bright red to orange, cheek bright red, keel bright red to orange, tip green and margin yellow to green; surface slightly waxy, slightly pubescent. *Pedicel* 0.5-0.8 cm, off white. *Fruit* drupe; immature fruit color off white, 3.94-4.30 mm x 5.55-6.84 mm; mature fruit not observed.

Distribution. West Indies, Guianas and Northern South America of the Atlantic. Widely cultivated in tropical countries

Habitat. At lowlands, 900 meters a.s.l. or lower, wet forest or thickets

Uses. Popular as outdoor ornamentals also cut-flower for floral arrangements (Arnold 2013; Maria et al. 2014), and often considered the typical ornamental *Heliconia* in Australia (Hintze 2014).

Specimen observed. Living collection of PBG with coll. number V.D.II.8 (H5).

Notes. *H. bihai* is considered as an invasive species (ISC, 2019). It can behave as a pioneer species, able to rapidly invade and colonize open and disturbed areas forming monocultures and avoiding the establishment of other plant species (Kress 1990a; Andersson, 1998; ISC 2019a).

***Heliconia latispatha* Benth.**

Heliconia latispatha Benth., Bot. Voy. Sulphur: 170 (1846); Griggs, Bull. Torrey Bot. Club 30 (12): 651 (1903); Standley & Steyemark, Fl. Gua. 24 (III): 182 (1952);); Kress, Fl. Mesoam. 7 (1): 14 (Figure 1B)

Vernacular name. Expanded lobster claw, Golden lobster claw, some cultivars are recognized includes "Orange Gyro' (orange with a green keel), 'Distans' (mostly red with yellow at base, and yellow joining rachis), "Red-Yellow Gyro (red with a small area of gold at the base).

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 90-114 cm, green with large brown-purplish blotches, waxy. *Leaf* form musoid-type; leaf blade shape oblong, oblique at base and acuminate at apex, 62-78 cm x 22-26 cm (ratio 2.9-3.0), leaf upper color green and pale green at lower; midrib upper color green and light green with reddish blotches at lower; leaf surface not waxy and dull at upper, not waxy and shiny at lower,

slightly undulating, not ripped. *Petiole* 27-42 cm, light green, pubescent, waxy. *Ligule* color green with brown-red blotches. *Inflorescenc*e erect. *Peduncle* 29-33 cm, yellow to light green, glabrous. *Rachis* very flexuosus, 16-18 cm, yellow to light green, glabrous. *Bract* deeply boat-shaped, 5-7, spirally arranged, middle bracts 12-14 cm x 2.0-3.0 cm, appearance lax (distance between bracts 2.0-3.0 cm); color at base yellow and bright red at cheek and keel, tip light green; surface not waxy, glabrous. *Perianth* dominant color yellow to light green with green tip, 4.1-4.3 cm x 0.7-1.0 cm. *Pedicel* 0.5-0.7 cm, cream to yellow. *Fruit* drupe; immature fruit 3.00-8.80 mm x 4.10-4.45 mm, yellow to green; mature fruit not observed.

Distribution. Mexico, Central America, to South America. Widely cultivated in tropical countries

Habitat. This species is found frequently along road cuts, forest edges, abundant in open secondary forests and recently open areas at altitudes 0-1000 m. Grow well in full sun to half shade and potentially become weeds.

Uses. Widely cultivated as ornamentals both garden ornamental and cut flower. As reported by Hintze (2014), it has long-lasting inflorescences which can last up to 9 months without losing their color.

Specimens observed. Living collections of PBG with coll. number V.D.II.19 (H3), V.D.II.20 (H6), V.D.II.15 (H9), V.D.II.25 (H13), V.D.II.24 (H16), V.D.II.14 (H17).

***Heliconia wagneriana* Petersen**

Heliconia wagneriana Petersen, Fl. Bras. 3 (3): 13 (1890); Kress, Fl. Mesoam 7 (1): 32 (2008) (Figure 1C)

Vernacular name. Easter heliconia.

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 61-79 cm, green with large brown blotches, very waxy. *Leaf* form musoid-type; leaf blade shape narrowly oblong, oblique at base and acuminate at apex, 90-114 cm x 21-26 cm (ratio 4.2-5.4); leaf upper color green, pale green at lower; midrib green with reddish line on ventral, lower color light green; leaf surface not waxy and shiny on both sides, very undulating, not ripped. *Petiole* 36-48 cm, green, glabrous, very waxy. Ligule color green with brown blotches. *Inflorescence* erect. *Peduncle* short, 2.0-3.0 cm, green, glabrous. *Rachis* slightly flexuosus, 17-18 cm, green, glabrous. *Bract* deeply boat-shaped, 5-6, distichous arrangement, size 11-13 cm x width 3.0-3.2 cm, appearance compact (distance between bracts 0.5-1.5 cm); color at base light green to green, cheek pale red, keel green, tip light green; surface not waxy, glabrous. *Perianth* dominant color yellow to green with green tip, middle bracts 4.3-4.5 cm x 0.7-1.0 cm. *Pedicel* 0.5-0.8 cm, off white to light green. *Fruit* drupe; immature fruit 7.30-9.00 mm x 4.00-5.00 mm, yellow to green; mature fruit not observed.

Distribution. Central America, Colombia, Ecuador, Atlantic to Pacific coasts. Widely cultivated in tropical countries.

Habitat. Common and abundant in open secondary forests in wet lowlands at altitudes 0-700 m. It was thriving in full sun and in spite of being a winter blooming species.

Uses. Widely cultivated as both garden ornamental and cut flower. The inflorescences can last up to 9 months without losing their color, while for cut-flower can last up to 3 weeks (Criley and Sakai 1997).

Specimens observed. Living collections of PBG with coll. number V.D.II.4 (H1), V.D.II.6 (H4).

Notes. According to ISC (2019b), *H. wagneriana* is included as invasive. It could invade the forest edges, disturbed or burnt habitats and lower montane rainforest (Krauss et al. 2008).

***Griggsia* Andersson**

Subgen. *Griggsia* Andersson (Figure 1-D-E-F)

Key characters. *Inflorescence* pendent, with *bract* inverted claw or boat-shaped.

***Heliconia chartacea* Lane ex Barreiros**

Heliconia chartacea Lane ex Barreiros, Revista Brasileira de Biologia 32: 205-207 (1972) (Figure 1D)

Vernacular name. Sexy pink heliconia.

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 150-194 cm, green with small brown blotches, waxy. *Leaf* form musoid-type; leaf blade shape narrowly oblong, rounded at base and acuminate at apex, 98-115 cm x 24-25 cm (ratio 4.1-4.6); leaf upper color green and pale green at lower; midrib upper color green and lower color light gree; leaf surface not waxy and shiny on both sides, not undulating, very ripped. *Petiole* 45-47 cm, green, glabrous, medium waxy, with green ligule. *Inflorescence* pendent. *Peduncle* 8-24 cm, pink, glabrous. *Rachis* slightly flexuosus, length 46-61 cm, pink, glabrous. *Bract* inverted boat-shaped, 9-14, spirally arranged, middle bracts 7.5-12 cm x 2.5-3.5 cm, appearance lax (distance between bracts 3-3.5 cm); color at base, cheek, and keel pale pink, with yellow to light green at tip and margin; surface slightly waxy, glabrous. *Perianth* dominant color yellow to green, tip green, 4.5-5 cm x 0.7-0.8 cm. *Pedicel* 2.0-3.3 cm, cream to pale yellow. *Fruit* drupe; immature fruit 8.21-8.62 mm x 5.33-5.55 mm, pale yellow; mature fruit not observed.

Distribution. Amazon Basin, Guianas to Ecuador. Widely cultivated in tropical countries.

Habitat. A common upland species of disturbed sites, young secondary forest, and abandoned cultivation, and is often found near human habitation.

Uses. cultivated as ornamental garden plants and as cut flowers for large arrangements. Easy to manage in the garden. All year round flowering, inflorescences long-lasting (Hintze 2014; Pereira et al. 2015; Loges et al. 2016).

Specimen observed. Living collection of PBG with coll. number V.D.II.26 (H15).

***Heliconia collinsiana* Griggs**

Heliconia collinsiana Griggs, Bull. Torrey Bot. Club 30: 648 (1903); Standley & Steyemark, Fl. Gua. Vol. 24 (III): 182 (1952); Kress, J. Arnold Arbor. 62 (2): 255-256 (1981), J. Arnold Arbor. 65: 482 (1984), Fl. Mesoam. 7 (1): 7 (2008) (Figure 1E)

Vernacular name. Red collinsiana.

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 149-175 cm, green with small

brown blotches, waxy. *Leaf* form musoid-type; leaf blade shape oblong, rounded at base and acuminate at apex, size 75-84 cm x 34-37 cm (ratio 2.2-2.3); leaf upper color green and pale green at lower; midrib upper color green and light green at lower with reddish line on central; leaf surface not waxy and shiny on upper side, waxy on lower side, not undulating, slightly ripped. *Petiole* 28-38 cm, green, glabrous, not waxy. *Ligule* color green with brown-red blotches. *Inflorescence* pendent. Peduncle 18-38 cm, bright red, slighly pubescent. *Rachis* slightly flexuosus, 25-41 cm, bright red, slighly pubescent. *Bract* inverted boat-shaped, 8-10, spirally arranged, middle bracts 11-13 cm x 3.0-3.5 cm, appearance lax (distance between bracts 3-6 cm); color at base, cheek, keel and tip red-orange; surface waxy particularly at apex, glabrous. *Perianth* dominant color yellow to orange, tip orange, 5-5.5 cm x 0.5-0.7 cm. *Pedicel* 1.5-2.5 cm, light green. *Fruit* drupe; immature fruit 8.85-9.45 mm x 7.10-7.70 mm, yellow; mature fruit not observed.

Distribution. Guatemala, Mexico to Central America. Widely cultivated in tropical countries.

Habitat. Type locality habitat in river bank. Found at forested slopes and open secondary growth, altitudes 50-1900 m. a.s.l.

Uses. cultivated as ornamental garden plants and as cut flowers for large arrangements. The species easy to maintain in the garden (Hintze 2014; Pereira et al. 2015; Loges et al. 2016)

Specimen observed. Living collection of PBG with coll. number V.D.II.12 (H11).

***Heliconia rostrata* Ruiz & Pav.**

Heliconia rostrata Ruiz & Pav., Fl. Peruv. 3: 71. pi. 305 (1803), Standley & Steyemark, Fl. Gua. 24 (III): 184 (1952) (Figure 1.F)

Vernacular name. Parrot's beak, Hanging heliconia, Hanging lobster claw, Painted lobster claw.

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 150-200 cm, green with large dark brown blotches, not waxy. *Leaf* form musoid-type; leaf blade shape narrowly oblong, rounded at base and acuminate at apex, 95-108 cm x 19-22 cm (ratio 4.9-5.0); leaf upper color dark green and pale green at lower; midrib upper color green and light green at lower; leaf surface not waxy and shiny on both sides, slightly undulating and ripped. *Petiole* 36-42 cm, green, pubescent and medium waxy. *Ligule* color green with dark brown blotches. *Inflorescence* pendent. *Peduncle* 36-42 cm, red, very hairy (velvety). *Rachis* straight, 35-53 cm, red, very hairy (velvet). *Bract* shape inverted claw-shaped, like a lobster claw, 8-21, distichous arrangement, middle bracts 6.5-7.5 cm x 3.0-4.0 cm, appearance compact (distance between bracts 1.5-2.0 cm); color at base, cheek and keel red scarlet, tip yellow to green, and margin yellow to green; surface very hairy (velvety), not waxy. *Perianth* dominant color cream to yellow with yellow tip, 3.8-4.5 cm x 0.5-1.1 cm. *Pedicel* 1.1-1.72 cm, off white. *Fruit* drupe; immature fruit 8.11-9.45 mm x 3.56-3.74 mm, off white to yellow; mature fruit not observed.

Distribution. Guatemala, Honduras, along the Atlantic coast to Panama and southward to Peru, Ecuador, and Brazil. Widely cultivated in tropical countries.

Habitat. The species frequently found at low elevation, along seasonally flooded river banks.

Uses. as ornamental plants in gardens and landscaped areas. This species is less tolerant of salt and wind exposure than some of other *Heliconia*. The inflorescence may be cut for indoor decoration where they last for several weeks (Arnold 2013; Hintze 2014; Pereira et al. 2015; Loges et al. 2016).

Specimens examined. Living collection of PBG with coll. number V.D.II.11 (H12).

***Stenochlamys* (Baker) Schum.**

Subgen. *Stenochlamys* (Baker) Schum. (Figure 1.G-H-I)

Key characters. *Inflorescence* erect, with *bracts* shallow-spathed and narrow shaped.

***Heliconia metallica* Planch. & Linden ex Hook**

Heliconia metallica Planch. & Linden ex Hook, Bot. Mag. 88, t. 5315 (1862), Kress, Fl. Mesoam. 7 (1): 20 (2008), Backer & Bakhuisen van den Brink III, 206: 40 (1968) (Figure 1.G)

Vernacular name. Shining bird of paradise.

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 125-167 cm, green with large brown-purplish, waxy. *Leaf* form cannoid-type; leaf blade shape narrowly oblong, acute at base and acuminate at apex, 98-112 cm x 22.5-26 cm (ratio 4.3-4.4); leaf upper color dark green purplish and light green purplish at lower, midrib upper color light green purplish at lower, waxy on upper side; leaf surface not waxy and dull on lower side, slightly undulating, not ripped. *Petiole* short, 1-5 cm, green, pubescent, not waxy. *Ligule* color brown and dry. *Inflorescence* erect. *Peduncle* 30-45 cm, light green, glabrous. *Rachis* slightly flexuosus, 9-13 cm, light green, glabrous. *Bract* shallow and narrow boat-shaped, 4-6, distichously arranged, middle bracts 5.0-6.5 cm x 1.1-1.8 cm, appearance lax (distance between bracts 2.3-2.6 cm); color light green at base, cheek green, keel light green, tip cream, and margin light brown and dry; surface not waxy, glabrous. *Perianth* dominant color red rose with white to light green tip, 4.1-4.5 cm x 0.6-0.8 cm. *Pedicel* 0.9-1.1 cm, off white to light green. *Fruit* drupe, 6.75-7.65 mm x 6.51-9.65 mm, immature fruit light green to green and blue metallic when ripe.

Distribution. Central America to Colombia, Venezuela, Ecuador, Peru, Bolivia, to Brazil. Widely cultivated in tropical countries.

Habitat. Commonly found in light gaps and margins of seasonally dry evergreen forests, at altitudes 0-1200 m.

Uses. It is useful as a garden plant, more for its beautiful purplish foliage than its inflorescences.

Specimen observed. Living collection of PBG with coll. number V.D.II.7 (H14).

***Heliconia hirsuta* L.f.**

Heliconia hirsuta L.f., Suppl. Pl.: 158 (1781); Griggs, Bull. Torrey Bot. Club 42 (6): 330 (1915); Kress, Fl. Mesoam. 7 (1): 11 (2008) (Figure 1H)

Vernacular name. not available. In their native country, it is commonly known by its cultivar name such as Peru, Chumaniana, Costa Flores, etc.

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 143-225 cm, green with small dark brown blotches, waxy. *Leaf* form zingiberoid-type; leaf blade shape oblong, rounded at base and acuminate at apex, 31-38 cm x 10.5-13 cm (ratio 2.8-3.4); leaf upper color green and pale green at lower, midrib upper and lower color light green; leaf surface not waxy and shiny on both sides, slightly undulating, not ripping. *Petiole* very short, 0.5-0.9 cm, green, pubescent, waxy. *Ligule* color green with dark brown blotches. *Inflorescence* erect. *Peduncle* 15-26 cm, yellow to light green, pubescent. *Rachis* straight, 4.0-4.5 cm, yellow to light green, pubescent. *Bract* shallow and narrow boat-shaped, 4-5, distichously arranged, middle bracts 7.6-11.5 cm x 0.8-1.3 cm, appearance compact (distance between bracts 1.1-1.9 cm); color at base, cheek, and keel pale yellow, with light green tip; surface waxy and pubescent. *Perianth* dominant color yellow with dark green spot on tip, 2.8-3.3 cm x 0.4-0.6 cm. *Pedicel* 1.1-2.0 cm, pale yellow. *Fruit* drupe, 6.42-7.70 mm x 3.91-6.42 mm, immature fruit off white to yellow and blue metallic when ripe.

Distribution. Central America to South America, Panama, Colombia, Venezuela, Guianas, Ecuador, Perú, Bolivia, Brasil, Paraguay, Argentina, to Caribbean.

Habitat. Found at open forests, altitudes 0-1000 m.

Specimen observed. Living collection of PBG with coll. number V.D.II.28 (H2).

Uses. This species is cultivated as a garden ornamental and for cut-flowers.

***Heliconia psittacorum* L.f.**

Heliconia psittacorum L.f., Suppl. Pl.: 158 (1781); Standley & Steyemark, Fl. Gua. 24 (III): 184 (1952) (Figure 1I)

Vernacular name. Parrot's flower, Parrot's beak, Parakeet flower. There are many varieties available such as "Andromeda' (orange inflorescences), ‘Lady Di' (red bracts and yellow flowers), ‘Kathy' (orangish-red bracts and orange flowers) and ‘Strawberries' (strawberry-red bracts) and ‘Cream' (pale yellow flowers).

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 59-90 cm, green with large brown blotches, waxy. *Leaf* form musoid-type; leaf blade shape very narrowly oblong, rounded at base and acute at apex, 36-56 cm x 8.0-10.5 cm (ratio 5.3-6.0); leaf color green on both sides, midrib light green on both sides; leaf surface not waxy and shiny on both sides, slightly undulating, not ripped. *Petiole* 10-29 cm, green, glabrous, waxy. *Ligule* color green with brown blotches. *Inflorescence* erect. *Peduncle* 40-60 cm, orange, glabrous. *Rachis* straight, 5.0-5.8 cm, orange, glabrous. *Bract* shallow and narrow boat-shaped, 3-4 distichously arranged, size 6.0-8.5 cm x 0.4-1 cm, appearance lax (distance between bracts 2.5-3.0 cm); color on base and cheek orange, keel reddish orange, tip yellowish and margin yellowish; surface slightly waxy, glabrous. *Perianth* dominant color orange with dark green spot, size 4.7-5.5 cm x 0.5-0.8 cm. *Pedicel* length 1.1-1,6 cm, orange. *Fruit* drupe; immature fruit 6.91-7.25 mm x 4.12-4.75 mm, orange; mature fruit not observed.

Distribution. Guatemala, Honduras, Panama, to South America. Very widely cultivated in tropical countries.

Habitat. Found in wet forest of the Atlantic lowlands, 480 meters or lower.

Uses. H. psittacorum is the most popular of the small *Heliconia*, long lasting, heavy stems and foliages. The species is cultivated as a garden ornamental and commercially for cut-flowers. The species can become weedy, has a tendency to become invasive without proper management, and may become impossible to eradicate from the ground (Krauss et al. 2008; Arnold 2013; Hintze 2014).

Specimen observed. Living collection of PBG with coll. number V.D.II.2 (H7).

Hybrid

Hybrid (Figure 1.J)

Key characters. *Inflorescences* erect; *rachis* slightly flexuosus; *bract* shallow and narrow boat-shaped, distichous to spirally arranged.

***Heliconia psittacorum x Heliconia spathocircinata* Aristeg.**

Heliconia psittacorum x Heliconia spathocircinata Aristeg. cv. Golden Torch (Figure 1.J)

Vernacular name. Commonly known by its cultivar name, 'Golden Torch'.

Morphological description. Perennial rhizomatous herbs. *Pseudostem* height 124-133 cm, green with moderate brown-reddish blotches, not waxy. *Leaf* form musoid; leaf upper color green and pale green at lower, midrib light green on both sides; leaf surface not waxy and dull at upper and not waxy and shiny at lower, not undulating, not ripped. *Petiole* 19-34 cm, green, slightly pubescent, not waxy. *Ligule* color green with brown-red blotches. *Inflorescence* erect. *Peduncle* 19-52 cm, orange with green dots, glabrous. *Rachis* slightly flexuosus, 12-16 cm, orange with green dots, glabrous. *Bract* narrow boat-shaped, 4-7, distichous to spirally arranged, middle bracts 10-11 cm x 1.8-2.3 cm, appearance lax (distance between bracts 2.2-3.0 cm); color at base red to orange, cheek, keel and tip orange to golden yellow; surface not waxy, glabrous. *Perianth* dominant color orange to golden yellow with faint green spot, 4.9-5.2 cm x 0.4-0.5 cm. *Pedicel* 0.7-1.2 cm, green to yellow-orange. *Fruit* drupe, 7.40-7.90 mm x 4.56-6.00 mm, immature fruit yellow to orange and reddish orange when ripe.

Distribution. Guianas and South America. Now, very widely cultivated in tropical countries.

Habitat. Prefer on open areas at secondary forests and river banks.

Figure 1. Inflorescences of *Heliconia* spp. collections of Purwodadi Botanic Gardens, East Java, Indonesia: Subgen. *Heliconia* (A) *H. bihai*, (B) *H. latispatha*, and (C) *H. wagneriana*; Subgen. *Griggsia* (D) *H. chartacea*, (E) *H. collinsiana*, and (F) *H. rostrata*; Subgen. *Stenochlamys* (G) *H. metallica*, (H) *H. hirsuta*, and (I) *H. psittacorum*; Hybrid species (J) *H. psittacorum x H. spathocircinata* cv. Golden Torch.

Figure 2. Leaf form type of *Heliconia*: A. Musoid, B. Cannoid, and C. Zingiberoid

Uses. This hybrid species is cultivated as a garden ornamental and commercially for cut-flowers. It does not flower during cold weather. It has the potential to become rather invasive without proper management in the garden (Krauss et al. 2008; Arnold 2013; Hintze 2014).

Specimens examined. Living collections of PBG with coll. number V.D.II.17 (H8), V.D.II.16 (H10).

Notes. Ecologists have assumed that interspecific hybridization normally was rare, however, some hybrids have been identified, including *H.* x Golden Torch. The species is natural interspecific hybrid between parents species of *H. psittacorum* (subgen. *Stenochlamys*) x *H. spathocircinata* (subgen. *Heliconia*) (Kress 1983; Criley and Broschat 1992). *H.* x Golden Torch has intermediate characters of both parents; particularly in inflorescence. *H. psittacorum* has erect inflorescence and bracts distichously arranged (Figure 1.I), whilst *H. spathocircinata* has flexuosus inflorescence and bracts spirally arranged (iNaturalist.org 2019). Thus *H.* x Golden Torch has slightly flexuosus inflorescence with bracts distichously to spirally arranged. Bract boat-shaped and narrow but not too shallow like *H. psittacorum* also not too deep like *H. spathocircinata*. Further some variation within *H.* x Golden Torch cultivar also available (Rocha et al. 2010).

Morphological diversity of *Heliconia* spp. collection of PBG

Based on morphological characters, *Heliconia* species collection of PBG examined were showing high variability. According to the leaf form was classified into three types, *i.e.* (i) musoid - leaves similar to banana (*Musa*), leaves upright with long petioles (Figure 2.A), (ii) cannoid - leaves similar to *Canna*, leaves held obliquely with short to medium-length petioles (Figure 2.B), and (iii) zingiberoid - leaves similar to ginger (*Zingiber*), leaves more or less horizontal with short petioles (Figure 2.C). Most of the *Heliconia* species examined were musoid type include *H. bihai*, *H. wagneriana*, *H. rostrata*, *H. chartacea*, *H. collinsiana*, *H. latispatha*, *H. psittacorum*, and *H. psittacorum* × *H. spathocircinata* (Figure 2.A). Meanwhile *H. metallica* was cannoid (Figure 2.B), therefore it was included in sect. *Cannastrum*; and *H. hirsuta* was zingiberoid (Figure 2.C), so that it was included in sect. *Zingiberastrum*.

According to inflorescence form was categorized into (i) erect or upward, which consisted of most of the species (Figure 1.A-B-C-G-H-I), and (ii) pendent or hanging, which become synapomorphic characters of subgen. *Griggsia* comprised *H. chartacea*, *H. collinsiana*, and *H. rostrata* (Figure 1.D-E-F). Further, according to the bracts arrangement to the rachis was categorized into (i) distichous (bracts arranged on opposite sides in the same plane) includes *H. bihai*, *H. wagneriana*, *H. rostrata*, *H. metallica*, *H. hirsuta* and *H. psittacorum* (Figure 1.A-C-F-G-H-I); and (ii) spirally arranged includes *H. latispatha*, *H. chartacea* and *H. collinsiana* (Figure 1.B-D-E). Whilst, hybrid species *H x* Golden Torch has intermediate characters varied distichously to spirally arranged (Figure 1.J).

Heliconia species was clearly morphologically distinguished by its inflorescence characteristics particularly the bract shape and color. *H. wagneriana*, *H. bihai* and *H. latispatha* (subgen. *Heliconia*) are characterized by erect inflorescence; deeply cymbiform

(boat-shaped) bracts and greenish flowers on untwisted pedicels as suggested by Andersson (1981). *H. bihai* is described by its bright red-orange, yellow to green margins bracts (Figure 1.A). *H. latispatha* is described by red over most of the bract with small area of yellow at base (Figure 1.B). Further, *H. latispatha* examined here was possibly identified as Red-Yellow Gyro cultivars. Whilst, *H. wagneriana* is described by its pale red areas over most of the cheek and is surrounded by light green to green at base and along to the keel and tip (Figure 1.C). Among subgen. *Heliconia*, *H. latispatha* has flexuosus rachis and spirally arranged bracts included in sect. Tortex, whilst the others have straight rachis and distichously arranged bracts (sect. *Heliconia*).

Subgen. *Griggsia* comprised *H. collinsiana, H. rostrata, H. chartacea*; distinguished by its pendent inflorescences as synapomorphic characters (Kress 1990a). Each species was differentiated by its typical bracts shape and color. Bracts of *H. chartacea* and *H. collinsiana* have an inverted boat-shaped, while *H. rostrata* has the shape of an inverted claw. Further, bract color of *H. chartacea* has pale pink with yellow to green margin and slightly coated with a silvery wax (Figure 1.D); whilst *H. collinsiana* has continuous red-orange bract and very waxy particularly at apex (Figure 1.E); and *H. rostrata* has red scarlet with yellow and green-tinged margin and tip, very hairy like velvet (Figure 1.F).

Subgen. *Stenochlamys* comprised *H. metallica, H. hirsuta*, and *H. psittacorum.* They have very diverse morphology both vegetative and generative parts. In general, subgen. *Stenochlamys* was characterized by its erect inflorescence with shallow-spathed and narrow shaped bracts (Griggs 1915; Andersson 1981). *H. metallica* has cannoid leaf with less attractive inflorescence, it has green bract with perianth rosy red and white tip (Figure 1.G). Whilst, *H. hirsuta* has zingiberoid leaf type with variable bracts generally orange, basally becoming red distally, sometimes green and yellow (Kress 2008). The specimen *H. hirsuta* examined has light green to pale yellow bracts with light green tip and waxy (Figure 1.H). Although there are some cultivars available, but this specimen examined cannot be identified its cultivar name yet, need further study. Meanwhile, *H. psittacorum* has musoid leaf type and showing wide variability of flowers color, there are many varieties available for ornamental purposes. The specimen of *H. psittacorum* examined showed pale orange to reddish bract with perianth reddish orange with black at distal part (Figure 1.I). It was possibly identified as Andromeda cultivar.

Golden Torch cultivar is considered a naturally hybrid species from *H. psittacorum* (subgen. *Stenochlamys*) and *H. spathocircinata* (subgen. *Heliconia*) (Kress 1983; Criley and Broschat 1992). Its characteristic was resemblance of typical their both parents such as leaf musoid type, erect inflorescence, bract boat-shaped and narrow, distichously to spirally aranged, with attractive orange-golden yellow bract color (Figure 1.J). According to the *Heliconia* Society of Puerto Rico, there are several hybrid cultivars of *H. psittacorum* x *H. spathocircinata* such as Alan Carle, Baby Flame, Guyana, Keanae Red, etc. Further, within *H.* x Golden Torch cultivar also showed some variation which differentiated by its bract color such as Golden Torch Adrian (red on cheek and keel), Golden Torch Sunshine (yellow, red-orange at tip), Pink Golden Torch (pink), etc. (Rocha et al. 2010).

Genetic diversity of *Heliconia* spp. collection of PBG inferred by *rbc*L sequences

Amplification of the *rbc*L marker was successfully carried out to 21 specimens examined, including ingroup and outgroup (Table 1). Visualization on 1.5% agarose gel electrophoresis was shown by the presence of a specific DNA band in the sample lane at the length of approximately ±700 bp. Further, direct sequencing on *rbc*L amplicons resulted in DNA sequences with length of 709 bp to 721 bp. Based on Basic Local Alignment Search Tool (BLAST) on NCBI GenBank, all data DNA sequences were homologs with *rbc*L sequences of the species from Zingiberales order (similarity 96-99%). The full length of *rbc*L sequences is 1,400 bp (Smith et al. 1993; Newmaster et al. 2006); however, according to NCBI data, some *rbc*L markers available were produced shorter amplicons of 350 to 950 bp on Zingiberales, such as in *Musa* spp. 500-530 bp (Ning et al. 2009; Hiariej et al. 2015), *Heliconia* spp. 256-857 bp (Hollingsworth et al. 2009; Garcia-Robledo et al. 2013); *Zingiber* spp. 523-922 bp (Vinitha et al. 2014; Ardiyani et al. 2009); *etc.*

The total aligned and selected *rbc*L sequences of specimens examined were 704 bp. It shows high conservation level (0.932) with 656 nucleotides were considered monomorphic (invariable). Only about 33 positions of DNA sequences were considered as polymorphic which comprised of 18 singleton variable sites (site that presents only at one distinct taxa) and 15 parsimony informative sites (sites that contain at least two types of nucleotides and present at least twice). Whilst, 15 sites were considered as alignment gaps or missing data. The 18 singleton variable site positions comprised two variants (37, 50, 51, 113, 134, 155, 236, 240, 281, 422, 440, 485, 587, 596, 626, 633, 686) and three variants (512). The parsimony informative sites consisted 14 parsimony-informative sites two variants (10, 107, 197, 206, 251, 332, 397, 617, 624, 632, 642, 646, 668, 701) and 1 parsimony-informative sites four variants (9). Parsimony informative sites are useful for reconstructing phylogenetic tree, meanwhile, singleton variation are non-informative sites that cannot provide information about which are parsimonous tree (Ubaidillah and Sutrisno 2009). Nonetheless, the singleton variation sequences specific to certain species may be proposed as identification barcodes (Hapsari et al. 2018).

Haplotype diversity of *Heliconia* spp. collection of PBG inferred by *rbc*L sequences

The haplotype analysis of in-group (Heliconiaceae) and outgroup (Strelitziaceae and Musaceae) using network program resulted in twelve haplotypes with haplotype diversity of 0.8952 (Figure 3). It was indicated that they were shared set of specific DNA sequences of *rbc*L gene and inherited together from a common ancestor (Seltman et

al. 2003). Pairwise distance analysis result also confirms that they were shared high similarity genetic material of *rbc*L with very low genetic distance of 0.022 to 0.000 among ingroup and outgroup, whilst among ingroup were 0.019 to 0 (Table S1). Genetic distance is used to measure the differences in genetic structure between two populations/species at a particular gene locus. The minimum value of 0 occurs if genetic structure of two populations/species are identical, while the maximum value of 1 indicates that they do not share any genetic type (Finkeldey 2005). Similar haplotype characterization based on *rbc*L sequences was performed in *Musa troglodytarum* populations from Moluccas with outgroups, i.e. *Musa fehi* and *Ensete* spp. (Musaceae); it showed low haplotype diversity value of 0.57, about six haplogroups were derived out of 24 specimens analyzed. Likewise, the genetic distances within ingroup showed very low genetic distances of 0.000, whilst among in grup and outgroup (*Musa fehi* and *Ensete* spp.) 0.010 to 0.020 genetic distances (Hiariej et al. 2015; Hiariej 2017).

Interestingly, Heliconiaceae haplotypes were positioned as median vector between Strelitziaceae and Musaceae haplotypes (Figure 2), therefore this study was explained why Heliconiaceae has intermediate characters of both families Strelitziaceae and Musaceae, particularly in their leaves and inflorescences characteristics. Heliconiaceae haplotypes were separated with Musaceae haplotypes by 9-14 nucleotides, and with Strelitziaceae haplotypes by 8-11 nucleotides. The haplotypes separation were mostly due to point mutations. Further, the ingroup of *Heliconia* species was separated into eight haplotypes comprises of three haplogroups (Hap 5, 6, 10) and five individual haplotypes (Hap 7, 8, 9, 10, 12) in which interconnected with median vector (Figure 3).

Haplogroup 5 was consists of *H. wagneriana*, *H. hirsuta*, *H. psittacorum*, and *H. x* Golden Torch with 0 genetic distance or genetically identical (Figure 3, Table S1). It was on the contrary of their morphological appearance which clearly has high variability (Figure 1.C-H-I-J), since they came from different subgen. *Heliconia*, *Stenochlamys*, and hybrid respectively. Further, *H. metallica* was separated in different haplotype due to single point mutation of nucleotide number 626. From Pendulae section, *H. rostrata* was considered genetically identical with *H. chartacea* and clustered in Haplogroup 11, whilst *H. collinsiana* was separated in Haplotype 10. differs by 6 nucleotides with genetic distance 0.009 (Figure 3, Table S1).

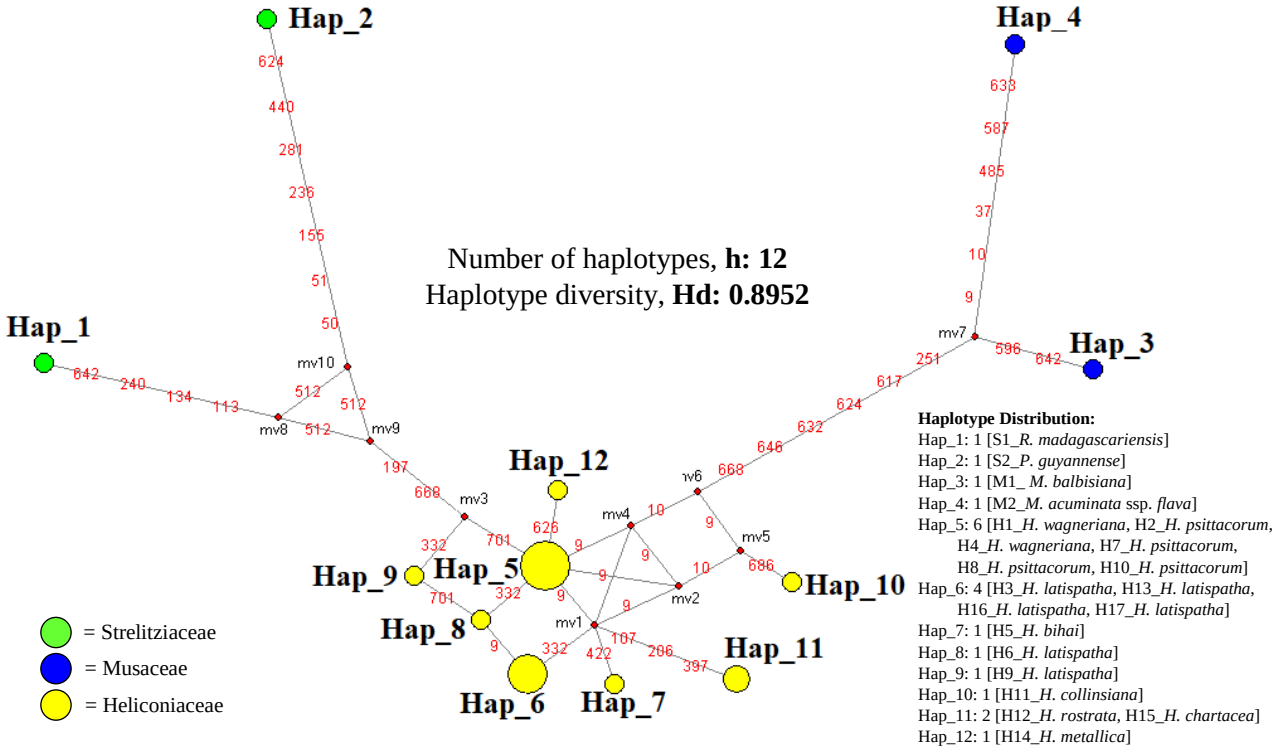


Figure 3. Haplotype map of in-group (Heliconiaceae) and outgroup (Strelitziaceae and Musaceae) inferred by *rbc*L sequences

Six specimens of *H. latispatha* were separated into 3 haplotypes (Hap 6, 8, 9) which differs in two nucleotides due to mutations, i.e. number 9 (T→A) and 701 (A→G) (Figure 3). *H. latispatha* which grouped in Haplotype 6 includes specimen codes H3, H13, H16 and H17 were considered genetically identical therefore for ex-situ conservation efficiency suggested to be eliminated and chosen just one living accession. Meanwhile, *H. metallica* and *H. bihai* were formed its own haplotype. Haplotype *H. metallica* (Hap 12) was closely related to Haplotype 5 by a nucleotide number 626 (A→T) at genetic distance 0.001, whereas haplotype of *H. bihai* (Hap 7) was closely related and connected by median vector to haplotype 5 and 6, which differs by every 2 nucleotides (Figure 3).

Phylogenetic of *Heliconia* spp. collection of PBG inferred by *rbc*L sequences

Phylogenetic analysis of the ingroup species inferred by *rbc*Lsequences data showed generally low bootstrap support (58-65), 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), and closely related to Musaceae and Strelitziaceae. Meanwhile, the separation of the outgroup Musaceae was supported by strong bootstraps (>95) and Strelitziaceae was supported by low to moderate bootstraps (63-81) (Figure 4.A-B-C). The higher of bootstrap values (70-100) suggest higher confidence level of the phylogenetic trees while the lower bootstrap values have high possibility of branching rearrangement (Kress 2002). Hence, the phylogenetic trees of Heliconiaceae in this study have high possibility in branching changes. However, the topology of phylogenetic tree resulted by Neighbour-Joining (NJ) algorithm has the best grouping with the highest bootstrap value of 65 (Figure 4.A) and be able to explain the relationship among species of *Heliconia* compared to Maximum Parsimony (MP) and Maximum Likelihood (ML) with same low bootstrap values of 58 (Figure 4.B-C).

The phylogenetic trees using both MP and ML algorithms produced polytomy in trees topology of ingroup *Heliconia* spp. Further, the separations of deeper branches (sections) were unclear, although there are some distinct clustering patterns (Figure 3.A-B). Previous genetic study using *rbc*L in *M. troglodytarum* populations resulted in phylogenetic tree with moderate bootstrap value (72) and polytomy topology (Hiariej et al. 2015). Polytomy is a branch that has more than two groups of lineage probably caused by evolution that occurred simultaneously at the same time which caused the uncertainty of phylogenetics (Kuhn et al. 2011). Polytomy trees of Heliconiaceae in this study confirm that *rbc*L marker was less suitable for the study of phylogeny among closely related species of single genus *Heliconia*. It was supported by previous genetic evolutionary studies on plants such as Fagaceae (Frascaria et al. 1993), Arecaceae (Hahn 2002); Poaceae (Hasegawa et al. 2009), Magnoliaceae (Huan et al. 2018), etc. which revealed that *rbc*L gene was evolved at very slow rate and not enough variability among species, therefore less powerful to differentiate close related species at lower taxa. However, *rbc*L sequences combined with other sequences from chloroplast genome (such as *mat*K, *trn*H-*psb*A, *trn*L-F, *rps*16, *rpo*B, etc.) generated phylogenetic trees with higher variability and bootstrap value of phylogenetic trees compared to *rbc*L data alone (Dong et al. 2012; Chen et al. 2015).

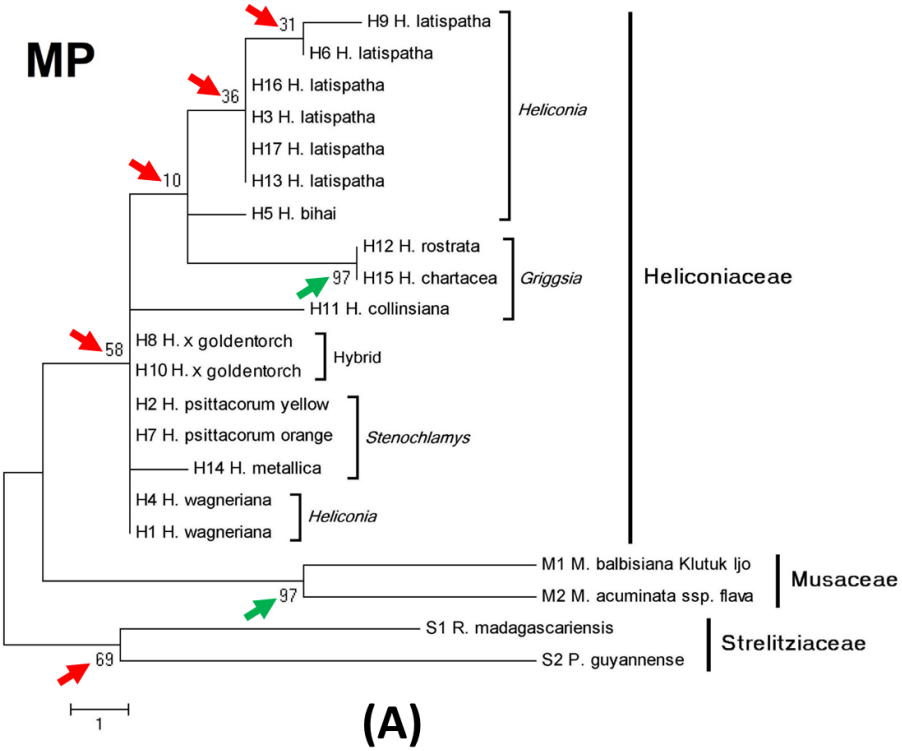
Tree topology of NJ algorithm was separated into two clades following its subgen. classification; with better bootstraps support (65) although still considered as low (Figure 4.C). Clade 1 consists of subgen. *Heliconia* (*H. latispatha* and *H. bihai*), and *Griggsia* (*H. rostrata*, *H. chartacea* and *H. collinsiana*) and Clade 2 comprises of subgen. *Stenochlamys* (*H. metallica*, *H. hirsuta*, and *H. psittacorum*), *Heliconia* (*H. wagneriana*) and also hybrid species (Figure 4.C). Therefore, based on *rbc*L sequences provide evidence that subgen. *Heliconia* is not monophyletic in opposed to previous *Heliconia* genetic relationship study by Marouelli et al. (2010) based on RAPD markers which shows that *subgen.* Heliconia was monophyletic. However, this result may be due to the lacking number of *Heliconia* species (subgen. *Heliconia*) examined.

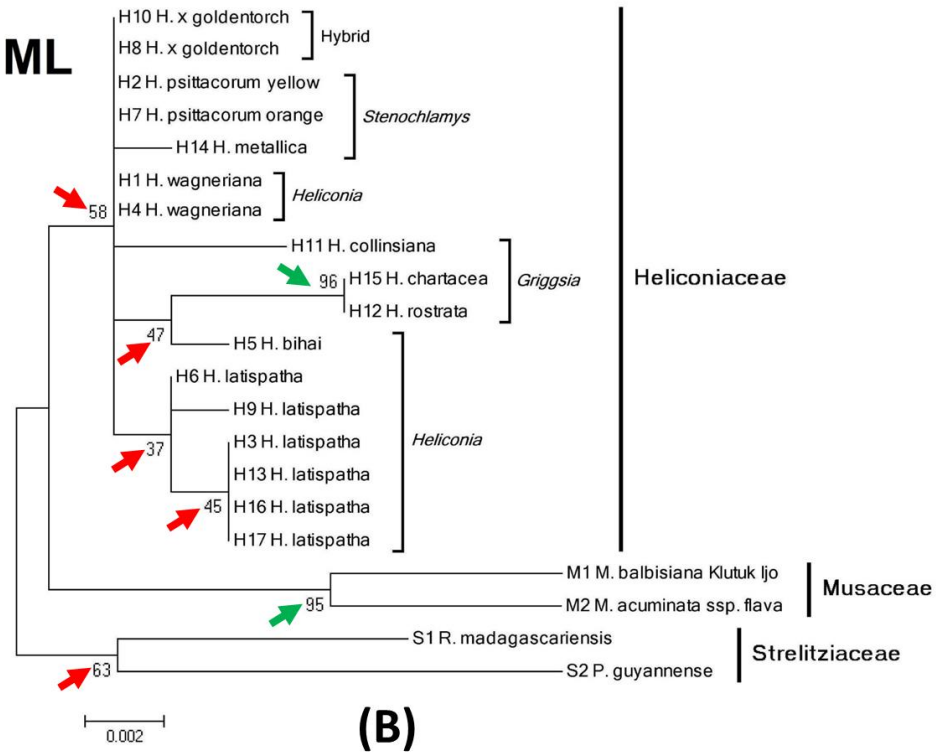
Further, the subgen. separation and deeper branching (section separation) of Clade 1 was not consistent and low in bootstraps. *H. collinsiana* was not clustered with other members of subgen. *Griggsia*, and became root of the subclades (Figure 4.C). Whilst, *H. rostrata* and *H. chartacea* of the subgen. *Griggsia* were consistently clustered with strong bootstraps (96-97) at all algorithms (Figure 4.A-B-C) and 0.000 genetic distance (Table S1). Likewise, *H. bihai* was not clustered with other members of subgen. *Heliconia* but *Griggsia*. Meanwhile, position of six *H. latispatha* specimens was consistently clustered in a subclade with low bootstrap (36-38) and genetic distance 0.003 to 0.000 at Clade 1 (Figure 4.C, Table S1).

The separations of Clade 2 were considered polytomy and low in bootstraps. *H. wagneriana* (subgen. *Heliconia*) was nested, at genetic distance 0.000 with subgen. *Stenochlamys*. Within subgen. Stenochlamys, *H. hirsuta* was considered identical to *H. psittacorum*, while *H. metallica* was separated at genetic distance 0.001 (Figure 4.C, Table S1). Further, this study confirms that specimens of the hybrid species *H. psittacorum* x *H. spathocircinata* cv. Golden Torch was nested close related to subgen. *Stenochlamys* where *H. psittacorum* specimens (a parental) clustered as suggested by previous study by Isaza et al. (2012) using AFLP markers. They were also considered genetically identical with genetic distance 0.000.

Implications for conservation and prospects

The diversity assessment both morphology and genetic have important implications for the successful conservation both in-situ and ex-situ and sustainable use. It provides baseline information of correct identity, which prevents duplication and omissions, and further identifies priorities for conservation. It also provides information that can be accessed and required for further development to improve the livelihoods (Leadlay and Jury 2006; Ibrahim et al. 2010).


MP
31
H9 H. latispatha
H6 H. latispatha
36
H16 H. latispatha
H3 H. latispatha
H17 H. latispatha
H13 H. latispatha
10
H5 H. bihai
Heliconia
H12 H. rostrata
97
H15 H. chartacea
Griggsia
H11 H. collinsiana
Heliconiaceae
58
H8 H. x goldentorch
H10 H. x goldentorch
Hybrid
H2 H. psittacorum yellow
H7 H. psittacorum orange
Stenochlamys
H14 H. metallica
H4 H. wagneriana
H1 H. wagneriana
Heliconia
M1 M. balbisiana Klutuk Ijo
M2 M. acuminata ssp. flava
Musaceae
97
S1 R. madagascariensis
S2 P. guyannense
Strelitziaceae
69
1
(A)


ML
H10 H. x goldentorch
H8 H. x goldentorch
Hybrid
H2 H. psittacorum yellow
H7 H. psittacorum orange
Stenochlamys
H14 H. metallica
H1 H. wagneriana
H4 H. wagneriana
Heliconia
58
H11 H. collinsiana
96
H15 H. chartacea
H12 H. rostrata
Griggsia
Heliconiaceae
47
H5 H. bihai
H6 H. latispatha
H9 H. latispatha
37
H3 H. latispatha
H13 H. latispatha
45
H16 H. latispatha
H17 H. latispatha
Heliconia
M1 M. balbisiana Klutuk Ijo
M2 M. acuminata ssp. flava
Musaceae
95
S1 R. madagascariensis
S2 P. guyannense
Strelitziaceae
63
0.002
(B)

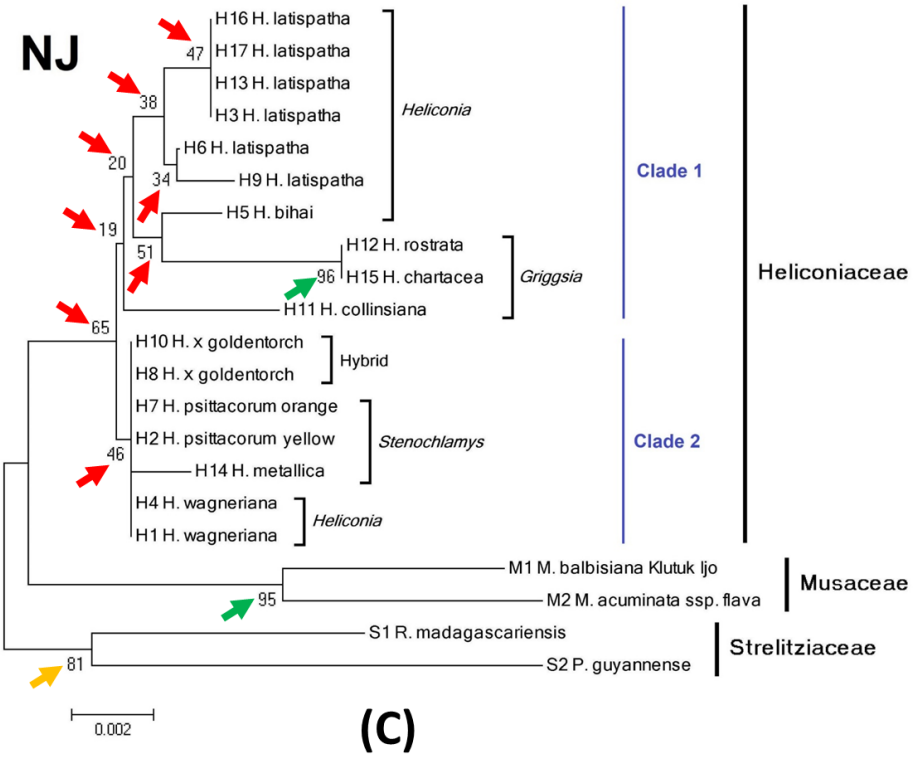


Figure 4. Phylogenetic trees of Heliconiaceae using different algorithms: A. Maximum Parsimony (MP), B. Maximum Likelihood (ML) and C. Neighbour-Joining (NJ). Notes: red arrow = branch with low bootstrap, yellow arrow = branch with moderate bootstrap, and green arrow = branch with high bootstrap

In terms of ex-situ conservation, if resources are limited, any identical specimens (genetic distance 0) both morphology and molecular should be chosen one of them as a representative collection. In this study; *H. wagneriana* specimen codes H1 and H4, also *H.* x Golden Torch specimen codes H8 and H10 were considered identical both morphology and genetic, therefore suggested to be eliminated and chosen just one living accession. Likewise, *H. latispatha* specimen codes H3, H13, H16, and H17 were considered morphologically and genetically identical so that should be chosen one of them. Whereas, *H. latispatha* specimen codes H6 and H9 are still necessary to be conserved; although they were morphologically identical but genetically variable.

Heliconia, in terms of in-situ conservation on its natural habitats, ex-situ conservation and for exotic ornamentals in the garden, some species examined were considered weedy, and has a tendency to become invasive without proper management, therefore, need for attention. *H. bihai* and *H. wagneriana* were included as invasive species. It is able to invade and colonize open and disturbed areas rapidly, also has an allelopathic which can inhibit the establishment of other plant species (Krauss et al. 2008; ISC 2019a, 2019b). *H. latispatha*, *H. psittacorum* and *H.* x Golden Torch were also potential as weeds and invasive (Booth 2010; Arnold 2013; Hintze 2014); since they grow rapidly, adaptive in damp locations and thriving of full sun, difficult to control once established in the environment. Periodic de-suckering is important to control the plant spreadings. Proper management practices are also suggested to be applied in ex-situ collection to prevent overlapping of the clumps between living accessions.

The *rbc*L sequences data of 21 specimens examined (Heliconiaceae, Musaceae and Strelitziaceae) have been deposited to the National Center for Biotechnology Information (NCBI) with GenBank accessions numbers MK238283 to MK238303 (Table 1). It will allow the bio-informatic data of *Heliconia* genetic diversity collection of PBG well documented and preserved, also provide intellectual property protection for further global purposes.

In Indonesia, *Heliconia* has become increasingly popular as an ornamental plant both outdoor in the garden and indoor as cut-flower. According to data from Ministry of Agriculture RI (2018), there was an increased of *Heliconia* stalk production as cut flower about 27.36% in 2017 over 2016, reached 1.38 million stalks. In general, consumers demand *Heliconia* inflorescence with characteristics of attractive, bright and colorful bracts also long-lasting. Some *Heliconia* species are excellent choices to be grown outdoors planted as ornamental in the garden, roadsides, border/fence, etc. combined with other plants. Further, with a great visual impact, *Heliconia* as cut-flower integrates with great ease in tropical styles of floral arrangements for bouquets, decoration at weddings, hotels,

offices, etc. from the simplest to the most sophisticated compositions (Maria et al. 2014). Most of the *Heliconia* species examined here are suitable for garden plants as well as cut flowers. *Heliconia* species with pendent inflorescence are generally easy to maintain in the garden (Loges et al. 2016). While, *H. metallica* is preferable for garden plant, more for its beautiful purplish foliage than its inflorescences which less attractive. Some *Heliconia* species have inflorescence characteristics which more durable than others. *H. latispatha* and *H. wagneriana*, *H. psittacorum*, *H.* x Golden Torch, and *H. rostrata* were considered long lasting up to 9 months without losing its color, and may cut for indoor decoration for several weeks lasting (Sultana and Hassan 2008; Arnold 2013, Loges et al. 2016). Further study on inflorescence longevity, care, and handling also preservation for cut-flower of the *Heliconia* species are suggested to be conducted.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Purwodadi Botanic Garden (PBG), Indonesian Institute of Sciences for providing the living plant materials studied and Indonesia Endowment Fund for Education, Ministry of Finance, Republic of Indonesia for funding the molecular part of this study with Graduate Research Fellowship grant number PRJ-541/LPDP/2013. Sincere thanks were also addressed to Plant Physiology Laboratory of Biology Department, Brawijaya University for the molecular and genetic facilities. Great appreciation to Ahmad Masrum (Coordinator of the living collections, PBG), Matrani (Plant identification, PBG), Patmiati (Librarian, PBG), laboratory staff and students for all the technical assistant and valuable discussions during the study.

REFERENCES

Andersson L. 1981. Revision of *Heliconia* sect. *Heliconia* (Musaceae). Nordic J Bot 1 (6): 759-784.

Andersson L. 1985. Revision of *Heliconia* subgen. *Stenochlamys* (Musaceae-Heliconioideae). Opera Bot 82: 1-124.

Andersson L. 1989. An evolutionary scenario for the genus *Heliconia*. In: Holm-Nielsen LB, Nielsen IC, Balslev H (eds.). Tropical Forests: Botanical Dynamics, Speciation and Diversity. Academic Press, London. pp. 173-184.

Andersson L. 1992. Revision of *Heliconia* subgen. *Taeniostrobus* and subgen. *Heliconia* (Musaceae - Heliconioideae). Opera Bot 111: 1-98.

Andersson L. 1998. Heliconiaceae. In: Kubitzki K. (eds) Flowering Plants. Monocotyledons. The Families and Genera of Vascular Plants, vol 4. Springer, Berlin, Heidelberg.

APG (The Angiosperm Phylogeny Group). 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot J Linn Soc 141: 399-436.

Ardiyani M, Newman M, Poulsen AD. 2009. A new species of *Zingiber* (Zingiberaceae) East of Wallace's line. Gard Bull Singapore 69 (2): 189-199.

Arnold MA. 2013. Landscape Plants for Texas and Environs. Stipes Publishing Co. Illinois, USA.

Backer CA, Bakhuizen van den Brink RC. 1968. Flora of Java (Spermatophytes Only). Vol. III. Wolters-Noordhoff, N.V. Groningen, Netherlands. pp. 39-40.

Barreiros HS. 1972. *Heliconia chartacea*. Revista Brasileira de Biologia 32: 205-207.

Bentham G. 1846. The Botanical Description. Bot Voy Sulphur. pp. 170.

Berry F, Kress JW. 1991. *Heliconia*. An Identification Guide. Smithsonian Institution Press.

Booth C. 2010. Climate change crop risks weedy date with destiny. Feral Herald Newsletter of the Invasive Species Council, Australia 26: 1-2.

CBOL (The Consortium for the Barcode of Life). 2009. A DNA barcode for land plants CBOL Plant Working Group1 Communicated. Proc Nati Acad Sci 106 (31): 12794-12797.

Chase MW, Soltis DE, Olmstead RG. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbc*L. Ann Missouri Bot Gard 80: 528-580.

Chen J, Zhao J, Erickson DL, Xia N, Kress WJ. 2015. Testing DNA barcodes in closely related species of *Curcuma* (Zingiberaceae) from Myanmar and China. Mole Eco Res 15: 337-348.

Clegg MT. 1993. Chloroplast gene sequences and the study of plant evolution. Proc Nati Acad Sci 90: 363-367.

Criley RA, Sakai WA. 1997. *Heliconia wargneriana* Petersen is a short-day plant. Hortsci 32 (6): 1044-1045.

Criley RA, Broschat TK. 1992. *Heliconia*: Botany and horticulture of a new floral crop Vol. 14. In: Janick J. Horticultural Reviews. John Wiley & Sons, New York.

Davis J, Stevenson DW, Petersen G, Seberg O, Campbell LM, Freudenstein JV, Goldman DH, Hardy CR, Michelangeli FA, Simmons MP, Specht CD, Vergara-Silva F, Gandolfo MA. 2004. A Phylogeny of the Monocots, as inferred from *rbc*L and *atp*A sequence variation, and a comparison of methods for calculating jackknife and bootstrap values. Syst Bot 29 (3): 467-510.

Dong W, Liu J, Yu J, Wang L, Zhou S. 2012. Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. PLoS ONE 7 (4): e35071. DOI: 10.1371/journal.pone.0035071.

Fauziah. 2017. Annual Report of Registration Unit, Purwodadi Botanic Gardens - Indonesian Institute of Sciences 2017. Purwodadi Botanic Gardens - Indonesian Institute of Sciences. Unpublished [Indonesian]

Finkeldey R. 2005. An Introduction To Tropical Forest Genetics: Molekuler Basic The Gene As A Function Unit. Institute Of Forest Genetics And Forest Tree Breeding. Germany.

Frascaria N, Maggia L, Michaud M, Bousquet J. 1993. The *rbc*L gene sequence from chestnut indicates a slow rate of evolution in the Fagaceae. Genome 36 (4): 668-671.

Garcia-Robledo C, Erickson D, Erwin T, Staines C, Kress WJ. 2013. Reconstructing a network of tropical plant-herbivore interactions using DNA Barcodes. PLoS ONE 8 (1): e52967. DOI: 10.1371/journal.pone.0052967

Griggs RF. 1903. On some species of *Heliconia*. Bull Torrey Bot Club 30 (12): 641-664.

Griggs RF. 1915. Some new species and varieties of *Bihai*. Bull Torrey Bot Club 42 (6): 315-330.

Guimarães WNR, Martins LSS, Castro CEF, Carvalho Filho JL, Loges V. 2014. *Heliconia* phenotypic diversity based on qualitative descriptors. Genet Mol Res 13 (2): 3128-3142.

Hahn WJ. 2002. A Molecular phylogenetic study of the Palmae (Arecaceae) based on *atp*B, rbcL, and 18S nrDNA sequences. Syst Biol 51 (1): 92-112.

Handique L, Parkash V, Hazarika AD. 2013. Phylogeny of *Musa paradisiaca*, *Ravenala madagascariensis* and *Heliconia rostrata* based on morphological, biochemical, amino acid sequences of *rbc*L protein and *mat*K DNA sequences. Int J Theor Appl Sci 5 (2): 69 -78.

Hapsari L. 2011. Dua dasawarsa koleksi pisang (Musaceae) di Kebun Raya Purwodadi (1990-2010). Berk Penel Hayati Edisi Khusus 5A: 147-151.

Hapsari L, Azrianingsih R, Arumingtyas EL. 2018. Genetic variability and relationship of banana cultivars (*Musa* L.) from East Java, Indonesia based on the Internal Transcribed Spacer region nrDNA sequences. J Trop Biol Conserv 15:101-120.

Hasegawa M, Zhong B, Zhong Y. 2009. Adaptive evolution of chloroplast genomes in ancestral grasses. Plant Signal Behav 4 (7): 623-624.

Hodge WH. 1941. The type species of *Heliconia*. Contrib Gray Herbarium Harvard Univ 135: 134-137.

Hollingsworth PM, Forrest LL, Spouge JL, Hajibabaei M, Ratnasingham S, et al. 2009. A DNA barcode for land plants. Proc Nati Acad Sci 106 (31): 12794-12797.

Hiariej A, Arumingtyas EL, Widoretno W, Azrianingsih R. 2015. Genetic kinship of tongkat langit banana (*Musa troglodytarum* L.) from Moluccas based on *rbc*L gene sequence. Indian J Genet 75 (4): 526-528.

Hiariej A. 2017. Characterization of rbcL gene sequences in tongkat langit banana (*Musa troglodytarum* L.) from Maluku. Res J Pharma Biol Chem Sci 8 (3): 249-253.

Hintze J. 2014. An Identification Picture Book for Heliconias and gingers For Cut Flower Growers. Jungle Plant & Flower Service. Darwin NT, Australia.

Huan HV, Trang HM, Toan NV. 2018. Identification of DNA barcode sequence and genetic relationship among some species of Magnolia family. Asian J Plant Sci 17: 56-64.

Ibrahim AA, Bakir MA, Khan HA, Al Farhan AH, Al Homaidan AA, Bahkali AH, Al Sadoon M, Shobrak M. 2010. A brief review of molecular techniques to assess plant diversity. Int J Mol Sci 11:2079-2096.

iNaturalist.org. 2019. iNaturalist Research-grade Observations. *Heliconia spathocircinata* Aristeg. observed in Brazil. Occurrence dataset https://doi.org/10.15468/ab3s5x accessed via GBIF.org on 2019-04-08. https://www.gbif.org/occurrence/2013734308.

Isaza L, Marulanda ML, López AM. 2012. Genetic diversity and molecular characterization of several *Heliconia* species in Colombia. Genet Mol Res 11 (4): 4552-4563.

ISC (Invasive Species Compendium). 2019a. Datasheet: *Heliconia bihai* (macaw flower). https://www.cabi.org/ISC/datasheet/109790. accessed on 1 February 2019.

ISC (Invasive Species Compendium). 2019b. Datasheet: *Heliconia wagneriana* (heliconia). https://www.cabi.org/ISC/datasheet/119450. accessed on 1 February 2019.

Krauss U, Seier M, Stewart J. 2008. Mitigating the Threats of Invasive Alien Species in the Insular Caribbean. Report on Project Development Grant (PPG) Stakeholder Meeting, GFL-2328-2740-4995. Piarco, Trinidad and Tobago: GEF, UNEP, CABI Caribbean and Latin America.

Kress WJ. 1981. New Central American Taxa of *Heliconia* (Heliconiaceae). J Arnold Arbor 62 (2): 243-260.

Kress WJ. 1983. Crossability Barriers in Neotropical *Heliconia*. Ann Bot 52 (2): 131-147.

Kress WJ. 1984. Systematics of Central American *Heliconia* (Heliconiaceae) with pendent inflorescences. J. Arnold Arbor 65: 483-485.

Kress J. 1990a. The diversity and distribution of *Heliconia* (Heliconiaceae) in Brazil. Acta Bot Bras 4 (1): 159-167.

Kress WJ. 1990b. The phylogeny and classification of the Zingiberales. Ann Missouri Bot Gard 77: 698-721.

Kress WJ, Beach JH. 1994. Flowering plant reproductive systems at La Selva Biological Station. In: McDade LA, Bawa KS, Hespenheide H, Hartshorn G (eds.), La Selva: Ecology and Natural History of a Neotropical Rain Forest. University of Chicago Press, Chicago. Pp. 161-182.

Kress WJ. 1995. Phylogeny of the Zingiberaceae: morphology and molecular. In: Rudall P, Cribb PJ, Cutler DF, Humphries CJ (eds.). Monocotyledons: systematics and evolution, Royal Botanic Gardens, Kew, UK, pp. 443-460.

Kress WJ, Prince L, Hahn WJ, Zimmer EA. 2001. Unraveling the evolutionary radiation of the families of the Zingiberales using morphological and molecular evidence. Syst Biol 50 (6): 926-944.

Kress WJ, Prince LM, Williams KJ. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. American J Bot 89: 1682-1696.

Kress WJ, Specht CD. 2006. The evolutionary and biogeographic origin and diversification of the Tropical Monocot Order Zingiberales. Aliso J Syst Evol Bot 22: 621-632.

Kress WJ, Erickson DL. 2007. A two-locus global DNA barcode for land plants: The coding *rbc*L gene complements the non-coding *trn*H-*psb*A spacer region. PLoS ONE 2 (6): e508. DOI: 10.1371/journal.pone.0000508

Kress WJ. 2008. Flora Mesoamericana. Heliconiaceae 7 (1): 1-33. Missouri Botanical Garden Press. Missouri, USA.

Kuhn TS, Mooers A, Thomas GH. 2011. A simple polytomy resolver for dated phylogenies. Methods Ecol Evol 2: 427-436.

Kumar PP, Yau JCK, Goh CJ. 1998. Genetic analyses of *Heliconia* species and cultivars with Randomly Amplified Polymorphic DNA (RAPD) markers. J America Soc Hort Sci123 (1): 91-97.

Leadlay E, Jury S. 2006. Taxonomy and Plant Conservation. The Cornerstone of the Conservation and The Sustainable Use of Plants. Cambridge University Press. Cambridge, UK.

Lestarini W, Matrani, Sulasmi, Trimanto, Fauziah, Fiqa AP. 2012. An Alphabetical List of Plant Species Cultivated in Purwodadi Botanic Gardens. Purwodadi Botanic Gardens - Indonesian Institute of Sciences. Pasuruan, East Java, Indonesia.

Linnaeus C. 1771. Mantissa Plantarum Altera. In:, Cramer J. 1961 Facsimile edition. Weinheim.

Linnaeus C. 1781. *Heliconia hirsuta* and *Heliconia psittacorum*. Suppl Pl 158-159. Orphanotrophe, Brunswig

Loges V, de Castro CEF, de Castro ACR, Goncalves C. 2016. Characteristics of pendent *Heliconia* for use in landscape and as cut flower. Ornamental Hort Campinas Sao Paulo 22 (3): 287-295.

Maria C, Singureanu V, Denisa H, Erszebet B. 2014. Heliconias - Novelties and applicability in floral art. J Hort Forest Biotech 18 (1): 22-27.

Marouelli LP, Inglis PW, Ferreira MA, Buso GSC. 2010. Genetic relationships among *Heliconia* (Heliconiaceae) species based on RAPD markers. Genet Mol Res 9 (3): 1377-1387.

Ministry of Agriculture Republic of Indonesia. 2018. Statistics of Agriculture 2018. Center of Data and Information System of Agriculture. Ministry of Agriculture, Republic of Indonesia. Electronic publication available at http://epublikasi.setjen.pertanian.go.id/epublikasi/StatistikPertanian/2018/Statistik%20Pertanian%202018/files/assets/basic-html/page223.html. Accessed on 30 March 2019. [Indonesian]

Newmaster SG, Fazekas AJ, Ragupathy S. 2006. DNA barcoding in land plants: evaluation of *rbc*L in a multigene tiered approach. Canadian J Bot-Revue Canadienne Bot 84: 335-341.

Ning S-P, Hakkinen M, Ge X-J. 2009. DNA barcoding in genus *Musa* L.: Testing the potential of proposed barcoding markers for species identification of *Musa*. Chinese Academy of Sciences, Guangzhou, Guangdong 510650, China. (Unpublished)

Ruiz Lopez H, Pavon J. 1802. Flora Peruviana, et Chilensis. Typis Gabrielis de Sancha, Madrid.

Pereira FRA, Moraes Filho RM, Martins LSS, Montarroyos AVV, Loges V. 2015. Genetic diversity and morphological characterization of half-sib families of *Heliconia bihai* L., *H. chartacea* Lane ex Barreiros, and *H. wagneriana* Peterson. Genet Mol Res 15 (2): gmr.15028003.

Petersen O G. 1890. Musaceae. in Martius C. Fl Bras 3 (3):1-27

Planchon JE, Linden JJ. 1862. *Heliconia metallica*. Bot Mag 88: t. 5315

Rocha FHA, Loges V, da Costa AS, de Aragão FAS, Santos VF. 2010. Genetic study with and interspecific hybrids. Crop Breed Appl Biotech 10: 282-288.

Schumann K. 1900. Musaceae. In: Engler A. (ed) Das Pfanzenreich IV.45: 1-45. Engelmann. Leipzig.

Seltman H, Roeder K, Devlin B. 2003. Evolutionary-based association analysis using haplotype data. Genet Epidemiol 25: 48-58.

Smith JF, Kress WJ, Zimmer EA. 1993. Phylogenetic analysis of the Zingiberales based on *rbc*L sequences. Ann Missouri Bot Gard 80 (3): 620-630.

Standley PC, Steyemark JA. 1952. Flora of Guatemala. Vol. 24, Part III pp. 178-190. Chicago Natural History Museum Press. Chicago, USA.

Sultana H, Hassan MDA. 2008. The genus *Heliconia* L. cultivated in Bangladesh. Bangladesh J Plant Taxon 15 (2): 141-153.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30 (12): 2725-2729.

Ubaidillah R, Sutrisno H. 2009. An Introduction to Biosystematic: theory and practice. LIPI Press. Jakarta. Indonesia. [Indonesian]

Vinitha MR, Kumar US, Aishwarya K, Sabu M, Thomas G. 2014. Prospects for discriminating Zingiberaceae species in India using DNA barcodes. J Integr Plant Biol 56 (8): 760-773.

Table S1. Matrix pairwise genetic distance based on *rbc*L sequences among ingroup (Heliconiaceae) and outgroup (Strelitziaceae and Musaceae)

Code	Species name	S1	S2	M1	M2	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17
S1	*R. madagascariensis*	0.000																				
S2	*P. guyannense*	0.018	0.000																			
M1	*M. balbisiana*	0.021	0.025	0.000																		
M2	*M. acuminata*	0.024	0.025	0.012	0.000																	
H1	*H. wagneriana*	0.012	0.016	0.015	0.015	0.000																
H2	*H. hirsuta*	0.012	0.016	0.015	0.015	0.000	0.000															
H3	*H. latispatha*	0.015	0.019	0.016	0.018	0.003	0.003	0.000														
H4	*H. wagneriana*	0.012	0.016	0.015	0.015	0.000	0.000	0.003	0.000													
H5	*H. bihai*	0.015	0.019	0.016	0.018	0.003	0.003	0.003	0.003	0.000												
H6	*H. latispatha*	0.013	0.018	0.016	0.016	0.001	0.001	0.001	0.001	0.004	0.000											
H7	*H. psittacorum*	0.012	0.016	0.015	0.015	0.000	0.000	0.003	0.000	0.003	0.001	0.000										
H8	*H.* x Goldentorch	0.012	0.016	0.015	0.015	0.000	0.000	0.003	0.000	0.003	0.001	0.000	0.000									
H9	*H. latispatha*	0.012	0.016	0.018	0.018	0.003	0.003	0.003	0.003	0.006	0.001	0.003	0.003	0.000								
H10	*H.* x Goldentorch	0.012	0.016	0.015	0.015	0.000	0.000	0.003	0.000	0.003	0.001	0.000	0.000	0.003	0.000							
H11	*H. collinsiana*	0.016	0.021	0.015	0.019	0.004	0.004	0.006	0.004	0.006	0.006	0.004	0.004	0.007	0.004	0.000						
H12	*H. rostrata*	0.018	0.022	0.019	0.021	0.006	0.006	0.006	0.006	0.006	0.007	0.006	0.006	0.009	0.006	0.009	0.000					
H13	*H. latispatha*	0.015	0.019	0.016	0.018	0.003	0.003	0.000	0.003	0.003	0.001	0.003	0.003	0.003	0.003	0.006	0.006	0.000				
H14	*H. metallica*	0.013	0.018	0.016	0.016	0.001	0.001	0.004	0.001	0.004	0.003	0.001	0.001	0.004	0.001	0.006	0.007	0.004	0.000			
H15	*H. chartacea*	0.018	0.022	0.019	0.021	0.006	0.006	0.006	0.006	0.006	0.007	0.006	0.006	0.009	0.006	0.009	0.000	0.006	0.007	0.000		
H16	*H. latispatha*	0.015	0.019	0.016	0.018	0.003	0.003	0.000	0.003	0.003	0.001	0.003	0.003	0.003	0.003	0.006	0.006	0.000	0.004	0.006	0.000	
H17	*H. latispatha*	0.015	0.019	0.016	0.018	0.003	0.003	0.000	0.003	0.003	0.001	0.003	0.003	0.003	0.003	0.006	0.006	0.000	0.004	0.006	0.000	0.000