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

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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 rbcL 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 rbcL marker subjected to 17 Heliconia spp. living collections of Purwodadi Botanic Garden (PBG) have been conducted. The rbcL 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 rbcL 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 rbcL 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, rbcL marker was considered too conserved thus less valuable for phylogenetic analysis at lower taxa among and within Heliconia spp. However, rbcL was suggested to distinguish at higher level taxa between closely related genus and above.

Keywords: Heliconia, identification, molecular marker, morphology, phylogenetic, rbcL.

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 been 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 *rbcL* 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 *rbcL* 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 *rbcL* gene variability in many plant species was considered too conserved, previous molecular studies reported *rbcL* 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 *rbcL* 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 bioinformatics 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 *Mus a balbisiana* (pisang klutuk ijo) and *Mus 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

<table>
<thead>
<tr>
<th>Code</th>
<th>Coll. number</th>
<th>Registration number</th>
<th>Species name</th>
<th>Subgen.*</th>
<th>Section*</th>
<th>Locality**</th>
<th>Genbank acc. number</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>V.D.II.4</td>
<td>Not available</td>
<td>Heliconia wagneriana Petersen</td>
<td>Heliconia</td>
<td>Heliconia Baker</td>
<td>East Java</td>
<td>MK238287</td>
</tr>
<tr>
<td>H2</td>
<td>V.D.II.28</td>
<td>P1997060328</td>
<td>Heliconia hirsuta L.f.</td>
<td>Stenochlamys Baker</td>
<td>Zingberastrum</td>
<td>Seram Island, Mollucas</td>
<td>MK238288</td>
</tr>
<tr>
<td>H3</td>
<td>V.D.II.19</td>
<td>P199507132</td>
<td>Heliconia latisspatha Benth.</td>
<td>Heliconia</td>
<td>Tortex L. Anderss.</td>
<td>Morotai Island, Mollucas</td>
<td>MK238289</td>
</tr>
<tr>
<td>H4</td>
<td>V.D.II.6</td>
<td>Not available</td>
<td>Heliconia wagneriana Petersen</td>
<td>Heliconia</td>
<td>Heliconia</td>
<td>East Java</td>
<td>MK238290</td>
</tr>
<tr>
<td>H5</td>
<td>V.D.II.8</td>
<td>P19780419</td>
<td>Heliconia biahi (L.) L.</td>
<td>Heliconia</td>
<td>Heliconia</td>
<td>West Java</td>
<td>MK238291</td>
</tr>
<tr>
<td>H6</td>
<td>V.D.II.20</td>
<td>P19940414</td>
<td>Heliconia latisspatha Benth.</td>
<td>Heliconia</td>
<td>Tortex L. Anderss.</td>
<td>West Java</td>
<td>MK238292</td>
</tr>
<tr>
<td>H7</td>
<td>V.D.II.2</td>
<td>Not available</td>
<td>Heliconia psittacorum L.f.</td>
<td>Stenochlamys Baker</td>
<td>Stenochlamys (Baker) Schum.</td>
<td>Malagasy</td>
<td>MK238293</td>
</tr>
<tr>
<td>H8</td>
<td>V.D.II.17</td>
<td>P19940411</td>
<td>Heliconia psittacorum x H. spathocircinata</td>
<td>Hybrid</td>
<td>-</td>
<td>West Java</td>
<td>MK238294</td>
</tr>
<tr>
<td>H9</td>
<td>V.D.II.15</td>
<td>P1994047</td>
<td>Heliconia latisspatha Benth.</td>
<td>Heliconia</td>
<td>Tortex L. Anderss.</td>
<td>West Java</td>
<td>MK238295</td>
</tr>
<tr>
<td>H10</td>
<td>V.D.II.16</td>
<td>P1994049</td>
<td>Heliconia psittacorum x H. spathocircinata</td>
<td>Hybrid</td>
<td>-</td>
<td>West Java</td>
<td>MK238296</td>
</tr>
<tr>
<td>H14</td>
<td>V.D.II.7</td>
<td>P19780418</td>
<td>Heliconia metallica Planch. &amp; Linden ex Hook.</td>
<td>Stenochlamys Baker</td>
<td>Cannastraum L. Anderss.</td>
<td>Brazil</td>
<td>MK238300</td>
</tr>
<tr>
<td>H15</td>
<td>V.D.II.26</td>
<td>P19950615</td>
<td>Heliconia chartacea Lane ex Barreiros</td>
<td>Griggsia L. Anderss.</td>
<td>Pendalae (Griggs) W.J. Kress, ined.</td>
<td>Brazil</td>
<td>MK238301</td>
</tr>
<tr>
<td>H17</td>
<td>V.D.II.14</td>
<td>P1994046</td>
<td>Heliconia latisspatha Benth.</td>
<td>Heliconia</td>
<td>Tortex L. Anderss.</td>
<td>West Java</td>
<td>MK238303</td>
</tr>
<tr>
<td>S1</td>
<td>V.D.II.1</td>
<td>P19810864</td>
<td>Ravenala madagascariensis Sonn.</td>
<td>-</td>
<td>-</td>
<td>Malagasy</td>
<td>MK238283</td>
</tr>
<tr>
<td>S2</td>
<td>V.D.II.13</td>
<td>P1981426</td>
<td>Phnakospermum guyannense (A.Rich.) Endl. ex Miq.</td>
<td>-</td>
<td>-</td>
<td>Brazil</td>
<td>MK238284</td>
</tr>
<tr>
<td>M1</td>
<td>XXIV.D.1</td>
<td>P1980041</td>
<td>Musa balbisiana Colla</td>
<td>-</td>
<td>Eumusa</td>
<td>Pasuruan, East Java</td>
<td>MK238285</td>
</tr>
<tr>
<td>M2</td>
<td>XXIV.A.29</td>
<td>P19810892</td>
<td>Musa acuminata var. flava (Ridl.) Nasution</td>
<td>-</td>
<td>Eumusa</td>
<td>Tuban, East Java</td>
<td>MK238286</td>
</tr>
</tbody>
</table>

Amplification of the rbcL marker was accomplished using primer pairs of rbcL-1F (5'-ATG TCA CCA CAA ACA GAA AC-3') and rbcL724R (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, USA (Taq DNA polymerase, 2x DreamTaq Green buffer, 0.4 mM each of dNTPs and 4 mM MgCl2), 3μL of 5 pmol each of forward and reverse primers, and 3μL of nuclease-free water. PCR thermal cycling program used for rbcL 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 °C, and extension for 45 seconds at 72 °C. Final extension carried out for 10 minutes at 72 °C. 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 Steyermark 1952); Flora of Java (Backer and Bakhuizen van den Brink 1968), Flora Mesoamericanica (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://biogodb.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 rbcL 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 spathochircinata cv. Golden Torch (Table 1). Fifteen Heliconia spp. examined (hybrid excluded) in this study were included in 3 subgenera i.e. Heliconia, Griggsia, and Stenochlamys. Further, each subgenus also divided into some sections such as Heliconia, Tortex, Pendulae, Rostratae, Stenochlamys, Zingberastrum 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 base. Leaves simple, distichous, petiolate, usually large; petiole i.e. long-length resembling bananas (musaoid), medium-length resembling canna (cannoid) and short-length resembling gingrs (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, leafflake, 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; 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 .................. 5
   b. Leaf blade upper and lower surface light green to green ................ H. metallica

3 a. Rachis straight, bract inverted claw-shaped, distichous, very hairy (velvety). .................. H. rostrata
   b. Rachis slightly flexuous, bract inverted boat-shaped, spiral, glabrous. ................ H. collinsiana

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. latisspatha
   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 flexuous, bracts spirally arranged, golden yellow color, perianth with distal faint green spot 

9 a. Bract color bright red to orange, with green tip and yellow to green margin .................. H. wagneriana
   b. Bract color green to pale red, with light green tip and green margin ........................ H. bihai

*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.


**Vernacular name.** Parrot’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 rippled. **Pctiole** 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 0.5 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 (HS)

**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 latisspatha* Benth.

*Heliconia latisspatha* Benth., Bot. Voy. Sulphur: 170 (1846); Griggs, Bull. Torrey Bot. Club 30 (12): 651 (1903); Standley & Steyermark, Fl. Guat. 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. Inflorescence erect. Peduncle 29-33 cm, yellow to light green, glabrous. Rachis very flexuous, 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 ornament 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.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 oblone, 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 green; leaf surface not waxy and shiny on both sides, not undulating, very rippled. Petiole 45-47 cm, green, glabrous, medium waxy, with green ligule. Inflorescence pendent. Peduncle 8-24 cm, pink, glabrous. Rachis slightly flexuous, 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 3.5-3.5 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, 4.5-5.5 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**


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 rippled. Petiole 28-38 cm, green, glabrous, not waxy. Ligule color green with brown-red blotches. Inflorescence pendent. Peduncle 18-38 cm, bright red, slightly pubescent. Rachis slightly flexuouss, 25-41 cm, bright red, slightly 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. Guat. 24 (III): 184 (1952) (Figure 1F)

**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 brown dark 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 rippled. 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 (velvety). 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 1G-H-I)

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

**Heliconia metallic da Planch. & Linden ex Hook**

*Heliconia metallic da* 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 1G)

**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 rippled. 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 flexuous, 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 zigzingeroid-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.2 (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' (orange-red bracts and orange flowers) and 'Strawberries' (strawberry-red bracts) and 'Cream' (pale yellow flowers).

**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 rippled. **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, distichously 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.** 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 1J)

**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 1J)

**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 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 rippled. **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.** Central America to South America, very widely cultivated in tropical countries.

**Habitat.** Prefer on open areas at secondary forests and river banks.
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 flexuous inflorescence and bracts spirally arranged (iNaturalist.org 2019). Thus H. x Golden Torch has slightly flexuous 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. Zingibernastrum.

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...
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 rbcL sequences**

Amplification of the rbcL 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 rbcL 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 rbcL sequences of the species from Zingiberales order (similarity 96-99%). The full length of rbcL sequences is 1,400 bp (Smith et al. 1993; Newman et al. 2006); however, according to NCBI data, some rbcL 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 rbcL 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 I 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 parsimonious tree (Ubaidillah and Sutrison 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 rbcL 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 rbcL 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 $rbcL$, 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 $rbcL$ 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 ingroup 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](image-url)
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. metallicla* and *H. bihai* were formed its own haplotype. Haplotype *H. metallicla* (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 rbcL sequences**

Phylogenetic analysis of the ingroup species inferred by rbcL sequences 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 rbcL 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 rbcL 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), Areceaceae (Hahn 2002), Poaceae (Hasegawa et al. 2009), Magnoliaceae (Huan et al. 2018), etc. which revealed that rbcL 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, rbcL sequences combined with other sequences from chloroplast genome (such as matK, trnH-psbA, trnL-F, rps16, rpoB, etc.) generated phylogenetic trees with higher variability and bootstrap value of phylogenetic trees compared to rbcL 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. metallicla, H. hirsuta,* and *H. psittacorum*), Heliconia (*H. wagneriana*) and also hybrid species (Figure 4.C). Therefore, based on rbcL 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 *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. metallicla* 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).
HAPSARI et al. – Species diversity and phylogenetic analysis of Heliconia spp.
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 *rbcL* 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.

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Table S1. Matrix pairwise genetic distance based on *rbcL* 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 | 0.000 | 0.000 |    |     |     |     |     |     |     |     |     |     |     |
| H6   | *H. latispatha*                | 0.013 | 0.018 | 0.016 | 0.016 | 0.001 | 0.001 | 0.001 | 0.004 | 0.000 | 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 | 0.000 | 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 | 0.000 | 0.000 |    |     |     |     |     |     |
| H11  | *H. collinsiana*               | 0.016 | 0.021 | 0.015 | 0.019 | 0.004 | 0.004 | 0.006 | 0.004 | 0.006 | 0.004 | 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 | 0.006 | 0.009 | 0.006 | 0.009 | 0.009 | 0.009 | 0.000 |    |     |     |     |
| H13  | *H. latispatha*                | 0.015 | 0.019 | 0.016 | 0.018 | 0.003 | 0.000 | 0.003 | 0.000 | 0.001 | 0.003 | 0.000 | 0.003 | 0.003 | 0.003 | 0.000 | 0.000 | 0.000 |    |     |     |
| H14  | *H. metallica*                 | 0.013 | 0.018 | 0.016 | 0.016 | 0.001 | 0.001 | 0.004 | 0.003 | 0.004 | 0.004 | 0.001 | 0.001 | 0.004 | 0.001 | 0.006 | 0.004 | 0.007 | 0.004 |    |     |
| H15  | *H. chartacea*                 | 0.018 | 0.022 | 0.019 | 0.021 | 0.006 | 0.006 | 0.006 | 0.006 | 0.007 | 0.006 | 0.006 | 0.009 | 0.006 | 0.009 | 0.000 | 0.000 | 0.006 | 0.007 | 0.000 |    |
| H16  | *H. latispatha*                | 0.015 | 0.019 | 0.016 | 0.018 | 0.003 | 0.000 | 0.003 | 0.000 | 0.003 | 0.001 | 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 | 0.000 | 0.003 | 0.000 | 0.003 | 0.001 | 0.003 | 0.003 | 0.003 | 0.006 | 0.006 | 0.000 | 0.004 | 0.006 | 0.000 | 0.000 |