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Essential Notes on the Passiflora incarnata-Agraulis vanillae insularis Relation

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Abstract

Context: The local vegetation was studied to identify elements that could host butterfly rearing (Lepidoptera: Papilionoidea) in the butterfly refuge at the Camaguey's Botanical Park

Aim: To unveil the nomenclature, taxonomy, phenotypical characterization, differences from akin taxa, distribution, and ethnobiological traits of a host plant (*Passiflora incarnata* L.), and one of the butterflies that lives in it (*Agraulis vanillae insularis* Maynard, 1889).

Methods: Field observation, work with biological collections, digital image processing, specimen identification through descriptive catalogs and analytical keys, experimental breeds in controlled conditions.

Results: The recorded history of *P. incarnata* L. in Cuba was reconstructed, and the origin of the germplasm found at the Camaguey's Botanical Park was elucidated. *A. vanillae insularis* Maynard (1889) demonstrated a potential as a host plant in Cuba. Different criteria were defined to contrast the two taxa with their akin. The feasibility of using the plant to rear butterflies for exhibition purposes was discussed.

Conclusions: Due to the frequency *P. incarnata* L. is cultivated in Cuba, it should be acknowledged as part of the economic flora of the nation. Considering that it acts as a host plant at the Camaguey's Botanical Park, and is capable of host the rearing of *A. vanillae insularis* Maynard (1889) butterflies, which have the potential to be included in the butterfly refuge under construction in the park.

Keywords: *Cuban economic flora*, *Passifloraceae*, *butterfly host plants*, *Heliconiinae*, *butterfly rearing.*

Introduction

The study of the local vegetation as a possible host for butterfly rearing in Camaguey resulted in the identification of a plant that acts as a host, whose presence in Cuba and its relation to the insects, produced scarce precise information in the literature consulted (Fuentes et al., 2000). Accordingly, this paper will deal with this issue.

The research was done as part of the project *A Butterfly refuge for sustainable management of butterflies at the Julio Antonio Mella People's Council*, coordinated by the Ignacio Agramonte Loynaz University of Camaguey. It aims to establish the scientific rationale and the proper technology for the work of the refuge, whose construction is in progress at the Camaguey's Botanical Park, under the fund provided by the Small Donations Program of the World Fund for the Environment.

Materials and Methods

For three years (2017-2020), *in situ* observations were conducted to determine the representativity of butterfly adults (Lepidoptera: Papilionoidea), as well as the larva cultivated at the Camaguey's Botanical Park. The measurements were made using a tape measure and a gauge caliper. Several digital images were taken and the plant's morphology, and insects at different stages were evaluated preliminarily.

Representative samples of the plant were collected and herborized, and then added to the Julián Acuña Galé Herbarium, the University of Camaguey (HIPC, according to Thiers, 2020). The species was

identified through comparisons using descriptors, keys, and images described by Fuentes et al. (2000); Goldman & MacDougal (2015); Costa et al. (2020).

The study also consulted the samples from digital herbariums, such as LINN, USF, FSU, and FLAS (Thiers, 2020). The Font Quer terminology was used to describe the plants.

The search for documentary evidence of the presence of this species in Cuba included the review of materials deposited in the herbariums, namely, HAC, HIPC and ULV (Thiers, 2020), and the bibliographic review and consultation to specialists associated with these institutions.

Field observations of the butterfly were conducted to detect eggs, larvae, and pupae, which could correspond to the target species. The capture of flying specimens was done with an entomological catching net. The collection also included specimens at different stages that were taken to experimental areas created at the Camaguey's Botanical Park and the zoology laboratory of The Ignacio Agramonte Loynaz University of Camaguey.

The potentially host plant was cultivated in a facility enclosed with a net, along with other plants where adult butterflies had been spotted drinking (nectariferous) nectar. Then, the conditions were created for butterfly rearing at all their life stages, including all the changes due to metamorphosis. Pictures were taken and the corresponding measurements and notes were processed.

The butterfly species were identified by comparing the images taken with the pictures appearing in specialized catalogs and specimens deposited at the McGuire Center for Lepidoptera and Biodiversity, Gainesville, Florida, the USA (MGCL, according to Bishop Museum, 2009). In the adult phase, the descriptions were based on Michener (1942); Alayo & Hernández (1987); Warren et al. (2016); Nuñez et al. (2020), and the sites: https://www.butterfliesofamerica.com/agraulis_vanillae_insularis_live1.htm (with dissected specimens) and https://www.butterfliesofamerica.com/agraulis_vanillae_insularis_live1.htm (living and flying individuals). The egg, larva, and pupa stages were studied according to Mari Mut (2015); Warren et al. (2016).

The information referred to the ecology and the life cycle of the butterfly species was taken from Riley (1975); Alayo & Hernández (1987); Fernández (2007); Lauranzón et al. (2013); Vásquez et al. (2017); Warren et al. (2017); Nuñez et al., 2020).

Results and discussion

The possibility that the butterfly refuge under construction includes this plant species to host the butterfly requires separate assessment of each organism, as well as their associations.

The host

Th plant with hosting conditions observed at the Camaguey's Botanical Park was identified as Passiflora incarnata L. (Passifloraceae). Its nomenclature, phenotypical characterization, distribution, and ethnobotanics are detailed below:

Passiflora incarnata L. Sp. Pl. 2: 959. 1753. (*nom. cons.*). Leptotype (Killip, Field Mus. Nat. Hist. Bot. ser. 19: 390. 1938): Herb. Linn. 1070.25 (LINN [photo!]). Figure 1.

Perennial, creeping plant that usually has additional roots off the main stem. Terete *stems*; young branches with short hairs. Alternate, simple, petiolate, three-lobe *leaves;* 3-5 x 0.5 mm linear-cetaceous stipules; eglandular, inconspicuous, deciduous; up to 4 cm pubescent, glandular *petiole*; protuberant, emerging *glands;* ± symmetrical limbs, 4-9(15) × 6-10 cm, deeply, lobulate; generally mid lobule longer than the sides; slightly serrated margin; bright face, tinily pubescent on the veins; pubescent back with thin prominent veins; absent abaxial nectareous. Solitary, large (3-5 x 4-6 cm), actinomorphic, conspicuous *flowers*; up to 5 mm pedicel; 5-6-glandulate, oblong ovoid bracts, of up to 1.5 x 1 cm, and cuneate and undulate to the apex, acuminate, denticulate on the edge; cupular hypanthium. Disepalous *Calix*; initially whitish 2 x 1 cm 5 sepals on the face, turning light violet when aged, oblong, and acute on the apex. Dipetalous *corolla;* 5 2-2.5 x 0,8-1 cm violet, oblong, and acute to cuspidate petals at the apex. Multiseriate *crown*, violaceous, sometimes lighter toward the apex. 5 *stamins*, 8 mm filaments, attached to the androgynophores in their lower side; linear-oblong anthers of up to 8 mm. Super sub-globous 5 mm, pubescent *ovary*; 1.2-1.5 cm gynophore; up to 8 mm stylus; clavate, ramified-3-stigma. Capsular berry, oblong-ovoid, 5 cm *fruit* that becomes yellow when mature. Numerous, arylate, compressed, reticulate to striate on the surface *seeds*. – Fl.: IV-X.

The plant rarely blossoms and bears fruit in the Cuban conditions (Fuentes & Alfonso, 1998), a phenomenon that Fuentes et al. (2000) attributed to the monoclonal origin of the germplasm spread in the country nowadays. Its reproduction is achieved vegetatively, by cuttings rooted around the stem.

It is native to the southeast of North America and Central America, and was introduced in South America and Europe (Costa et al., 2020). It has not

been reported in the West Indies (Acevedo & Strong, 2012), except for Cuba.

Common name: Pasiflora.

The presence of the species in Cuba was recorded initially by Richard (1850), which was later reproduced by Grisebach (1866) and Sauvalle (1873), who cited the former. The fact that the plant went unnoticed to other botanists who investigated the Cuban flora later Cuba (Gómez de la Maza, 1889 and 1897; Gómez de la Maza & Roig, 1916; Alain, 1953; Roig, 1965), led to the idea that the initial introduction, should it actually exist) did not prosper, and the plant never spread through the country.

In 1973, The Juan Roig Experimental Station of Medicinal Plants in San Antonio de los Banos, introduced the species from the Experimental Station of Medicinal Plants of Kobuleti, Georgia (Svanidze et al., 1974; Acosta & Granda, 1985; Fuentes et al., 2000). In the last two decades of the twentieth century, it was added to the list of fruit plants cultivated in Cuba (Cañizares, 1982; Esquivel et al., 1989; Esquivel et al., 1992). So far, no link between that record and the germplasm imported has been determined to have public health benefits.

Certainly, at the Juan Tomas Roy Experimental Station of Medicinal Plants, the species spread out to other provinces (Nápoles et al., 2007; Jacas et al., 2017). Today, several private and state farmers cultivate the plant intensively, probably in all the provinces to extract a raw drug from the foliage for use in the pharmaceutical industry.

Considering the information above, Passiflora incarnata L. should be added to the preliminary inventory of the vascular plants of Cuba (Greuter & Rankin, 2017). The clear allusion to that scientific name in Latin in the works of Richard (1850), Grisebach (1866), and Sauvalle (1873) provides sufficient grounds for the inclusion of the plant in that list, though as the plant has spread out, it deserves to be treated comprehensively as part of the economic flora of the Republic of Cuba.

The plants cultivated at the Camaguey's Botanical Park were brought directly from the Juan Tomas Roig Experimental Station of Medicinal Plants, in the 1990s, by Jesús Ávila Herrera, one of the authors of this paper. Nowadays, this species is also cultivated in the Previsora neighborhood, in the city.

Specimens spotted: Cuba, Camagüey. The Alvaro Barba Machado Agricultural Polytechnical Institute (21.35324 – 77.875602), cultivated plant I-2020, J. Ávila HPC-*12684* (HIPC). Previsora neighborhood (21.384811 – 77.936941), in self-supply green gardens III-2021, *R. González* and *J. Ávila, HPC-12683* (HIPC).

The fruit of Passiflora incarnata L. is consumed in different parts of the world, either fresh or processed industrially to produce juices and jam (Howell, 1976; Cervi, 1997). The species adds to other three of the same genus observed in Cuba (Greuter & Rankin, 2017, which classified as fruit plants (*P. quadrangularis* L., *P. maliformis* L. and *P. edulis* Sims). They can be contrasted using the following analytical key

1 Young quadrangular branches .....*P. quadrangularis*

1* More or less cylindrical young branches..... 2

2 Entire leaf lamina (unilobulate).....*P. maliformis*

2*Three-lobe leaf lamina..... 3

3 Several additional shoots off the main stem; thinly serrated leaf margin.....*P. incarnata*

3 Additional shoots off the main stem absent; markedly serrated leaf margin.....*P. edulis*

In numerous countries, Passiflora incarnata L. is used in traditional natural medicine, as the pharmaceutical industry sells root extracts, young stems, and aerial parts, which are crushed for oral use as tablets, capsules, pills, tinctures, and extracts (Nápoles et al., 2014; Jacas et al., 2017).

It can be used to treat anxiety, alcoholism, insomnia, cough, nervous and sleep disorders, hyperactivity, abstinence and menopause syndromes, irritability, migraine, and psychosomatic manifestations (Hernández et al., 2007; Grundmann et al., 2008; Rabanal & Castelo-Branco, 2009; Elsas et al., 2010; Pereira, 2014; Poconé et al., 2017).

The plant has demonstrated hypolipidemic, hyperglycemic, and anti-asthmatic effects (Pereira, 2014), and the extracts, mixed with the extracts of *P. alata* Curtiss, have a powerful inhibitory effect on the growth of tumor cells that cause acute lymphoblastic leukemia (Ozarowski et al., 2018).

Dhawan et al. (2004) demonstrated the presence of phenolic compounds, especially flavonoids, such as apigenin, campherol, 2-glucosyl apigenin, 2"-O-glucosyl-6-C-glucosyl apigenin, 6-β-D-glucopyranosyl-8-β-D-ribopyranosyl apigenin, isoorientin, isoorientin-2-O-β-xylo-pyranoside, isoscoparin-2"-O-glucoside, isoschaftoside, isovitexin, isovitexin-2"-O-β-glycopyranoside, luteolin, orientin, quercetin, schaftoside, svertisin and vitexin. Moreover, it contains maltol derivatives, raffinose-type carbohydrate, sucrose, D-fructose and

D-glycose, essential oils containing hexanol, benzylic alcohol, linalol, phenylethyl alcohol, 2-hydroxybenzic acid methyl ester, carvone, trans-anethole, eugenol, isoeugenol, β-ionone, α-bergamotol, and phytol.

The host

As a result of the search, the butterfly at Camaguey's Botanical Park spotted dwelling on Passiflora incarnata L. (Passifloraceae) was identified as a subspecies of *Agraulis vanillae* (Linnaeus, 1758), from the Nymphalidae, subfamily Heliconiinae. The species inhabits the American continent, from the US to Argentina, including the Caribbean (Alayo & Hernández, 1987). It comprises eight sub-species, among which, only A. vanillae insularis Maynard 1889 (Figure 2) is found in Cuba (Warren et al., 2017).

It nomenclature, phenotypical classification, distribution and ethnology are detailed below.

Agraulis vanillae insularis Michener Amer. Mus. Novelilates 1215: 2. 1942 = *Agraulis insularis* Maynard Contrib. to Sci., Newtonville, Mass., 1: 89. 1889 = *Dione vanillae insulari* Stichel Gen. Ins. 63: 19. 1907. Lectotipo (Lamas Rev. Per. Entom. 40: 119. 1997): Andrews Bahamas, 8-XII-1881, Maynar 16661 (MGCL [photo!]).

The adults have a span of 65-80 mm, the upper face of the wings are orange-reddish, very intense in the males and opaque in females. The front wing veins are well marked, with large black areas on the vein apexes, particularly Cu l and Cu 2. The two sexes have black marks, though more spread in the females; the lower face of the wings have irregular silver spots; the insects fly fast and actively. They lay 1-3 eggs. Helicoidal yellow or gray *egg* of up to 2 mm high. Eruciform-type *larva*, up to 4 cm, with alternate orange and black stripes, and protuberant black spines. Obtect and cremaster-suspended *pupa or chrysalid* of about 3 cm, yellow with slight brown motifs in the early stages, grayish when grown, similar to a dry leaf, having slight orange tones like rings in the abdomen, on which end they hang.

It lives in Cuba, the Bahamas, and the other West Indies, except the southeastern Caribbean.

Common name in Cuba: *Plateada* (silver) (Fernández & Minno, n/a.; Nuñez et al., 2020).

Agraulis vanillae insularis Maynard 1889 is one of the most abundant butterflies in the country, commonly observed in lawns and gardens, as well as in the open fields. In Cuba, the larva dwells on a plant species of genus *Passiflora* (Passifloraceae), whereas the adult consumes the nectar of *Asclepias curassavica* L. (Apocynaceae), *Bidens pilosa* L. var. *pilosa* (Asteraceae), *Dalechampia scandens* L. (Euphorbiaceae), *Calopogonium caeruleum* (Benth.) C. Wright (Fabaceae, Faboideae), *Ixora coccinea* L. (Rubiaceae) and *Bouchea prismática* (L.) Kuntze (Verbenaceae) (Fernández, 2007; Lauranzón et al., 2013).

Its presence in the Camaguey's Botanical Park (a macro institution that will provide a home to the butterfly refuge, the esthetic potential and availability of the proper host plants inside the flying area) validates the selection of Agraulis vanillae insularis Maynard (1889) among the group of butterfly species that will be reared in the new institution. Preliminary trials conducted as part of this research demonstrated that such aspiration is perfectly viable.

Host-guest relation

The capacity of *Passiflora* has been well-documented to host butterfly larvae and pupae, particularly the ones in the sub family Heliconiinae (Ryler, 1975; Alayo & Hernández, 1987; Fernández, 2007; Lauranzón et al., 2013; Vásquez et al., 2017; Martin, 2020; Nuñez et al., 2020).

The host-guest affinity is clear to the researchers who have studied this topic in Cuba. In generic terms, *Passiflora* L. has been acknowledged as the host of *Heliconius charitonia ramsdeni* Comstock & Brown 1950 (Fernández & Minno, n/a.; Lauranzón et al., 2013). Moreover, *P. capsularis* L. and *P. suberosa* L. have been recorded as the hosts of *Dryas iulia nudeola* (Bates, 1934), and *P. edulis* Sims. and *P. suberosa* L. of *Agraulis vanillae insularis* Maynard 1889 (Bates 1935; Fernández, 2007).

Internationally, P. incarnata L. has also been recorded as the host of *Heliconius charitonius* Comstock & Brown, 1950 (*Pasiflora incarnata* host plant, 2011) *and Agraulis vanillae* (Linnaeus) (Daniel, 2009; Martin, 2020), both from sub-family Heliconiinae.

Due to the limited distribution of P. incarnata L. in Cuba (excluding the scientific and production institutions), it is logical to think that its host condition for native butterfly species had not been referred to previously by the researchers who study this topic in the national territory. However, Fuentes et al. (2000) noted that Agraulis vanillae insularis Maynard 1889 is one of the pests that attack these plants. The authors added that the larvae feed from the leaves, causing damage at any time of the year and the raw drug collected from the plant for the industry loses properties.

The results of searches conducted at the Camaguey's Botanical Park have provided a more general approach than the merely economic side mentioned

by the same authors. Ecologically, adults were seen ovipositing on the leaves, and the presence of eggs, larvae, and pupae corroborate the thesis that P. incarnata L. is the host species of Agraulis vanillae insularis Maynard (1889) in Cuba.

This association between a native lepidoptera and a recently-introduced plant might seem curious. Although it is part of the new relations created after the introduction of exotic elements in a particular region (which necessarily entails a readjustment of the dynamic of systemic functioning of biodiversity). The truth is that this contact had already been recorded in areas near Cuba (Daniel, 2009).

Although the relation described above entails a threat to the production of biomass from P. incarnata L. in Cuba, which could be used in the pharmaceutical industry, it is an opportunity to rear Agraulis vanillae insularis Maynard 1889 under controlled conditions, as was demonstrated in experimental conditions in this research paper.

The planting of P. incarnata L. may be encouraged so it can act as the host of Agraulis vanillae insularis Maynard 1889. It is a plant that can be maintained in cultivation conditions, on which there is plenty of experience nationally and internationally (which is not inherent to other plant species that can assume the role of hosts, even from the same genus). The plant also produces abundant foliage (at least during the rainy season), so it has a high production potential. Furthermore, it is esthetically attractive, even in its vegetative state, but much more when it blossoms (which is rarely in Cuban conditions). Its creeping habits can be used to mask structures from the public.

P. incarnata L. can be cultivated inside the flying area of the butterfly refuge, and become a pillar to foster the entire life cycle of Agraulis vanillae insularis Maynard (1889). Its growth can be stimulated in the production area of host and nectariferous plants to generate: a) biomass for larval nutrition in lab conditions; b) eggs, which previously certified, could be transferred for controlled hatching; c) pupae that, after evaluation, can be exhibited to the public.

Conclusions

Nowadays, *P. incarnata* L. is frequently cultivated in Cuba, and it should be acknowledged as part of the economic flora of the nation.

P. incanata L. is the host plant of Agraulis vanillae insularis Maynard (1889) at the Camaguey's Botanical Park.

Agraulis vanillae insularis Maynard (1889) has a potential to be reared and exhibited at the butterfly refuge under construction at the Camaguey's Botanical Park.

P. incarnata L. growing should be encouraged so it can support the rearing of Agraulis vanillae insularis Maynard 1889 at the Camaguey's Botanical Park.

Author contribution statement

Julio C. Rifa Tellez: Bibliographic review, plant identification, overall redaction of the manuscript, photo composition.

Marisela Guerra Salcedo: Research planning, field sampling, bibliographic review, identification of the butterfly species, and redaction of parts of the text.

Isidro E. Méndez Santos: Conception of the manuscript's structure, bibliographic review, identification of the butterfly species, and redaction of parts of the text.

Jesús Ávila Herrera: Field sampling, identification, cultivation, and study of the plant and the life cycle of the butterfly. Final review of the manuscript.

Conflicts of interests

The authors declare there are no conflicts of interests.

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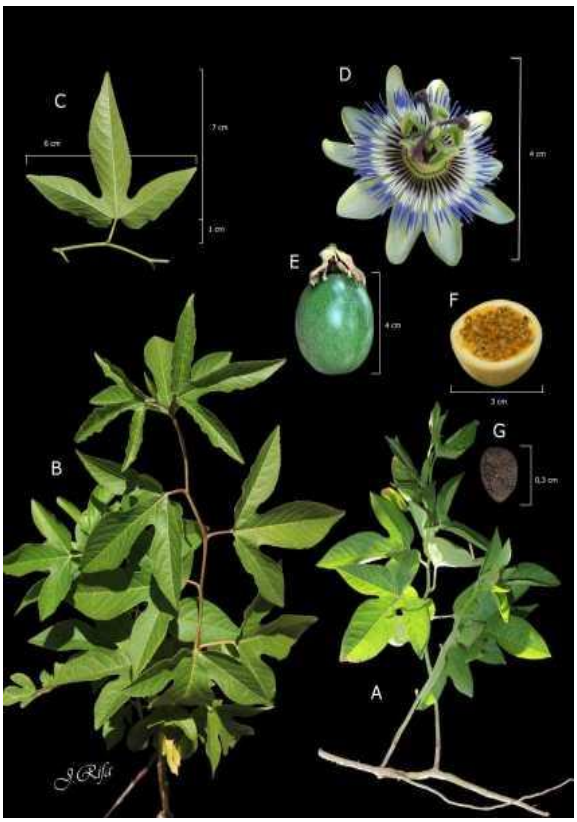

Figure 1. Passiflora incarnata L. A. Shoots coming out a lateral root. B. A branch with leaves with a face-top view. C. Leaves with a view of the reverse. D. Flower. E. Immature fruit. F. A transversal section of the mature fruit. G. Seed. A, B, and C. Photos taken by Roeris Gonzalez Sivilla. D and G, retrieved from https://hablemosdeflores.com/la-passiflora-incarnata-o-pasionaria/. retrieved from: https://www.youtube.com/watch?v=9bplhtbe008. F. Retrieved from: http://ventananatural.blogspot.com/2011/08/la-parchita-passiflora-incarnata.html

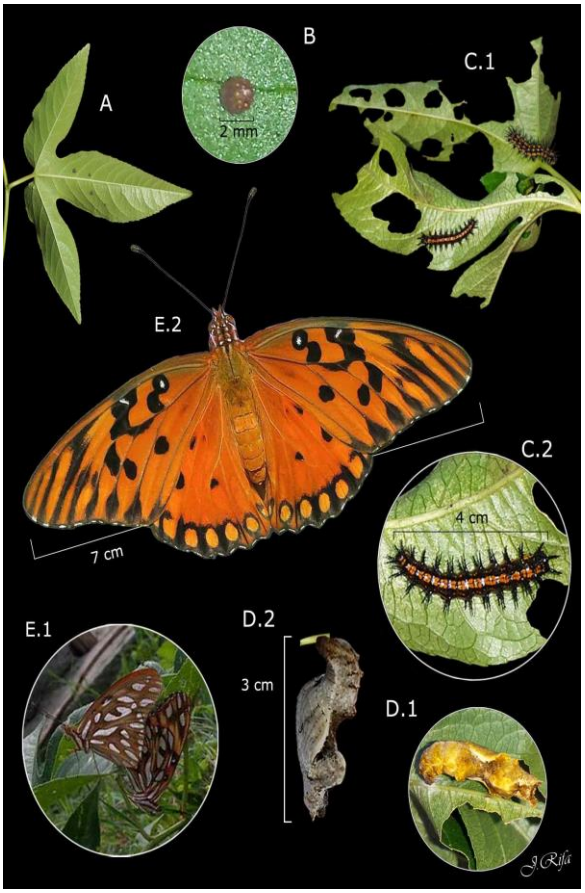

Figure 2. Agraulis vanillae insularis Maynard (1889) and its relation with Passiflora incarnata L. A. Leaves of *P. incarnata* with *A. vanillae insularis* eggs on the reverse. B. Egg of *A. vanillae insularis*. C.1. Larvae feeding from a leaf. C. 2 A Larva zoom in. D.1. Chrysalid in its initial stage of development. D.2. Chrysalid in its late stage of development. E.1 Copulating adults showing the lower side of their wings E.2. Adult showing the upper side of wings. Photos taken by Roeris Gonzalez Sivilla.