PHYLOGENY AND BIOGEOGRAPHY OF *HETEROSTYLUM* (DIPTERA: BOMBYLIIDAE): EVIDENCE FOR AN ANCIENT CARIBBEAN DIVERSIFICATION MODEL

Carlos José Einicker Lamas[1,4,*], Silvio Shigueo Nihei[2] , André Mallemont Cunha[3] and Márcia Souto Couri[3,4]

[1]Museu de Zoologia da Universidade de São Paulo, Avenida Nazaré, 481, 04263-000, Ipiranga, São Paulo, SP, Brazil
E-mail: einicker@usp.br

[2]Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Travessa 14, n. 101, 05508-900, Cidade Universitária, São Paulo, SP, Brazil
E-mail: silvionihei@gmail.com

[3]Museu Nacional, Quinta da Boa Vista, 20940-040, São Cristóvão, Rio de Janeiro, RJ, Brazil
E-mail: a_mcunha@yahoo.com.br, mcouri@terra.com.br

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*Corresponding author; E-mail: einicker@usp.br

Abstract

A cladistic and biogeographic analysis is presented of *Heterostylum* Macquart (Diptera: Bombyliidae), a New World bee-fly genus with 14 species. A phylogenetic hypothesis was obtained based on a data matrix with 27 characters, using the cladogram analysis and search program, TNT, and the matrix editing and tree viewing program, WINCLADA. Character states were polarized by tree rooting with the following outgroup taxa: *Toxophora aurea* Macquart, *Apiformyia australis* Yeates, *Triploechus novus* Williston, *T. bellus* Philippi, and *T. heteronevrus* Macquart. The monophyly of *Heterostylum* was well supported, and after successive weighting was applied, 2 major clades were found: a Nearctic clade including *H. robustum* (Osten Sacken), *H. helvolum* Hall & Evenhuis, *H. deani* Painter, *H. croceum* Painter, and *H. engelhardti* Painter, and a Neotropical clade with *H. haemorrhoicum* (Loew), *H. rufum* (Olivier), *H. evenhuisi* Cunha & Lamas, *H. maculipennis* Cunha & Lamas, *H. ferrugineum* (Fabricius), *H. hirsutum* (Thunberg), and *H. pallipes* Bigot. For the biogeographic analysis we derived an area cladogram based on the phylogenetic hypothesis obtained to analyze the distributional pattern and spatial diversification of *Heterostylum*. The divergence between Nearctic and Neotropical clades is associated with a spatial disjunction along the Mexican Transition Zone, which supports evidence that an ancient Caribbean event was mainly responsible for the diversification of major lineages of *Heterostylum*. This biogeographic scenario, as well as alternative scenarios, was also analyzed and discussed along with the results obtained from an event-based biogeographical analysis (DIVA).

Key Words: cladistic biogeography, dispersal-vicariance analysis, DIVA, Nearctic, Neotropical, systematics

Resumen

Se presenta un análisis cladístico y biogeográfico del género *Heterostylum* Macquart (Diptera: Bombyliidae), un género de mosca-abeja del Nuevo Mundo con 14 especies. Se obtuvo una hipótesis filogenética basado sobre una matriz de datos con 27 caracteres, utilizando el análisis de cladograma y un programa de búsqueda, TNT, y el programa de edición de matriz y de visualizar el árbol, WINCLADA. Los estados de los carácteres fueron polarizados por el árbol de enraizamiento con el siguiente taxa del grupo externo: *Toxophora aurea* Macquart, *Apiformyia australis* Yeates, *Triploechus novus* Williston, *T. bellus* Filipos y *T. heteronevrus* Macquart. La monofilia de *Heterostylum* fue bien apoyado, y después de aplicar parcialidad sucesiva, se encontraron 2 clados principales: el clan Neártica que incluye *H. robustum* (Osten Sacken), *H. helvolum* Hall & Evenhuis, *H. deani* Painter, *H. croceum* Painter, y *H. engelhardti* Painter, y el clados Neotropical con *H. haemorrhoicum* (Loew), *H. rufum* (Olivier), *H. evenhuisi* Cunha y Lamas, *H. maculipennis* Cunha y Lamas, *H. ferrugineum* (Fabricius), *H. hirsutum* (Thunberg), y *H. pallipes* Bigot. Para el análisis biogeográfico derivamos un clado-

grama de área basada en la hipótesis filogenética obtenida al analizar el patrón de distribución y la diversificación espacial de *Heterostylum*. La divergencia entre los clanes del Neártico y Neotropico se asocia con una disyunción espacial por la Zona de Transición Mexicana, que apoya la evidencia de que un evento antiguo del Caribe fue responsable principalmente por la diversificación de los principales linajes de *Heterostylum*. Este escenario biogeográfico, así como los escenarios alternativos, también fueron analizados y discutidos junto con los resultados obtenidos de un análisis biogeográfico basado en eventos (DIVA).

Palabras Clave: biogeografía cladística, análisis de dispersion-vicarianza, DIVA, Neártica, Neotropical, sistemática

The genus *Heterostylum* Macquart, 1848 contains medium-sized species (10-15 mm) characterized primarily by a robust body covered with long pile and by an indented hind margin of the eye. It presently contains 14 New World species, 9 of them occurring in the Neotropical region [*H. bicolor* (Loew), 1861; *H. duocolor* (Painter & Painter), 1974; *H. evenhuisi* Cunha & Lamas, 2005; *H. ferrugineum* (Fabricius), 1805; *H. haemorrhoicum* (Loew), 1863; *H. hirsutum* (Thunberg), 1827; *H. maculipennis* Cunha & Lamas, 2005; *H. pallipes* Bigot, 1892 and *H. rufum* (Olivier), 1789] and 5 in the Nearctic region [*H. croceum* Painter, 1930; *H. deani* Painter, 1930; *H. engelhardti* Painter, 1930; *H. helvolum* Hall & Evenhuis, 1981 and *H. robustum* (Osten Sacken), 1877].

Hull (1973) proposed 10 tribes for the Bombyliinae, and placed *Heterostylum* (along with *Triploechus* Edwards, *Efflatounia* Bezzi, and *Karakumia* Paramonov) in his Heterostyliini, based on the indented hind margin of the eye. Hall (1976) suggested morphological similarities between *Triploechus* (which occurs in North America and in South America, only west of the Andes) and *Heterostylum* (which occurs in South America and is recorded only east of the Andes).

Bowden (1985) proposed a new classification for the Bombyliinae, synonymized Heterostylini with Dischistini, and presented characters to distinguish the 2 largest tribes, Bombyliini and Dischistini. However, Yeates (1994) considered the characters provided by Bowden (1985) to support Dischistini as plesiomorphies, thus making the tribe paraphyletic, and to be used only for taxonomic convenience. As a result of Yeates's cladistic study, *Heterostylum* was transferred to the Bombyliini.

Cunha et al. (2007) revised *Heterostylum* and considered 14 species as valid. The authors redescribed the genus and species and also presented a key to all of them.

More recently, Yeates (2008) erected *Apiformyia*, a new genus from Australia, to include a single species *A. australis*. The author placed the genus among the Bombyliinae and pointed out its remarkable anatomical affinities with *Heterostylum*.

In the present study, a cladistic analysis of *Heterostylum* was carried out including 12 New World species. The distributional pattern and diversification of *Heterostylum* were analyzed through an area cladogram derived from the phylogenetic hypothesis. The historical context of divergent, non-overlapping clades in the area cladogram was discussed relied upon the geological history and the available molecular dating. Complementary analysis using an event-based method (DIVA) was performed to test and refine our proposed biogeographic hypothesis.

MATERIAL AND METHODS

The material studied is deposited in the collections of the Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ), Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP), Departamento de Zoologia, Universidade Federal do Paraná, Paraná, Brazil (DZUP), Museu Paraense Emílio Goeldi, Pará, Brazil (MPEG), National Museum of Natural History, Smithsonian Institution, Washington D.C., USA (USNM), and the Natural History Museum, London, UK (BMNH). Because no specimens of *A. australis*, *T. novus*, *H. engelhardti*, *H. helvolum*, and *H. robustum* were available for this study, their data were obtained from literature sources (Hall & Evenhuis 1981; Yeates 2008).

For the cladistic analysis we included 12 New World species of *Heterostylum* in the ingroup. The types of 2 species, *H. bicolor* and *H. duocolor*, are lost and no other specimen is known to be deposited in collections, and, because the original descriptions are insufficiently detailed to provide information for the great majority of characters, they were not included in the cladistic and biogeographic analyses. Only morphological characters of adults were used (Table 1) and all of them were treated as non-additive. The selected outgroup is composed by 3 species of *Triploechus*: one Nearctic (*T. novus* Williston, 1893) and 2 Andean species (*T. bellus* Philippi, 1865 and *T. heteronevrus* Macquart, 1850), one species of the Australasian genus *Apiformyia* (*A. australis* Yeates, 2008), and one species of *Toxophora* (*T. aurea* Macquart, 1848), which was used to root the tree. Based on morphological similarities, *Triploechus* is herein considered *a priori* the hypothesized sister-group

TABLE 1. CHARACTERS AND CHARACTER STATES USED IN THE CLADISTIC ANALYSIS OF *HETEROSTYLUM*.

Head:

1- Ocellar tubercle with a row of setae separating the anterior ocellus from the others: (0) absent; (1) present.

2- Color of scape: (0) same as pedicel and flagellum; (1) different from pedicel and flagellum.

3- Length of scape related to pedicel: (0) more than twice as long; (1) twice as long; (2) less than twice as long.

4- Length of flagellum related to scape + pedicel: (0) twice as long; (1) less than twice as long; (2) more than twice as long.

5- Color of palpus: (0) uniform; (1) apical portion darker than base.

6- Eyes with an indented hind margin: (0) absent; (1) present.

Thorax:

7- Length of head related to thorax: (0) narrower; (1) as wide as or wider.

8- Color of hairs on thorax: (0) entirely gold or yellow; (1) entirely dark brown; (2) gold on mesonotum and dark brown on pleura; (3) dark brown on mesonotum and yellowish brown on pleura.

9- Scutellum with dark-brown setae on posterior margin: (0) present; (1) absent.

10- Row of setae on the anterodorsal surface of femur III: (0) absent; (1) present.

11- Wing pigmentation: (0) beyond apical 1/3 of cell bm; (1) only at the base, never beyond basal 1/3 of cell bm; (2) only at the base, but surpassing the basal 1/3 of cell bm; (3) at base and around all veins.

12- Wing coloration: (0) unicolored; (1) bicolored.

13- Veins M_1 and R_5: (0) never merging at the point where R_4 arises; (1) merging near or at the point where R_4 arises.

14- Shape of vein R_1: (0) curved: (1) bent toward the superior margin.

15- Shape of vein R_4: (0) conspicuously sinuous; (1) slightly sinuous.

16- Anal cell: (0) opening in wing margin by ½ r-m crossvein length; (1) opening in wing margin by 2/3 r-m crossvein length; (2) opening in wing margin by the length of r-m crossvein; (3) closed before wing margin.

Abdomen:

17- Color of hairs on tergites: (0) predominantly light brown or yellow; (1) predominantly dark brown.

18- Color of hairs on sternite I: (0) light; (1) dark.

19- Pattern of coloration on tergites: (0) transverse stripes on at least one segment; (1) round spots; (2) a central longitudinal stripe; (3) absent.

20- Pattern of coloration of hair on abdominal dorsum: (0) with uniform coloration; (1) with apex darker than base.

21- Male genitalia with epiphallus: (0) absent; (1) present.

22- Length of distiphallus (aedeagus): (0) long, surpassing gonocoxal margins; (1) short, not surpassing gonocoxal margins.

23- Female genitalia with sand chamber: (0) absent; (1) present.

24- Acanthophorite spines: (0) absent; (1) present.

25- Length of sperm pump related to the spermathecae: (0) smaller; (1) longer.

26- Shape of furca: (0) "U" shaped; (1) "W" shaped.

27- Shape of spermathecae: (0) elliptical; (1) circular; (2) coiled.

of *Heterostylum*, and *A. australis* is the single species of an Australasian genus that shares similarities with *Heterostylum* in the conformation of the posterior eye margin and wing venation (Yeates 2008). Because the indentation of the posterior eye margin is a variable character in *A. australis*, not observed in all specimens (N. L. Evenhuis, pers. comm.), we coded it in the data matrix (Table 2) as polymorphic for this species.

The program TNT version 1.1 (Goloboff et al. 2007) was used for the cladogram search, and WINCLADA (Nixon 2002), version 1.00.08, for the matrix editing and the tree viewing and editing.

We applied 2 different character weighting schemes: equal and successive. The successive weighting approach (Carpenter 1988, 1994) is an iterative scheme that applies different weights to characters according to their performance or fitness [interpreted as phylogenetic reliability by Carpenter (1994)] in the initial analysis with equal weights. Character performance can be quantified by several character indices [*e.g.* consistency index (CI), retention index (RI) or

TABLE 2. DATA MATRIX OF THE CLADISTIC ANALYSIS OF *HETEROSTYLUM* MACQUART (? = MISSING DATA).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Toxophora aurea	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1	3	0	0	0	0	1	1	0	0	0	2
A. australis	0	0	1	0	0	0	0	0	1	0	1	0	0	1	1	0	0	0	2	0	0	1	0	0	0	0	0
T. bellus	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	-	3	0	1	1	1	1	?	?	?
T. novus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	?	0
T. heteronevrus	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	?	1	0	0	0	1	0	1	1	?	?	?
H. croceum	1	0	0	2	0	1	1	0	1	1	1	0	1	1	?	?	0	0	3	1	1	0	1	1	1	?	0
H. deani	1	0	0	2	0	1	1	0	1	1	1	0	1	1	0	2	0	0	0	0	1	0	1	1	1	0	0
H. engelhardti	1	1	0	2	0	1	1	0	1	1	1	0	1	1	?	?	0	0	3	1	1	1	1	1	1	?	0
H. evenhuisi	1	0	1	1	0	1	1	3	0	1	2	0	1	1	0	2	1	0	1	0	1	1	1	1	0	0	0
H. ferrugineum	1	1	1	1	0	1	1	0	0	1	1	0	1	1	1	1	0	0	2	0	1	1	1	1	1	0	1
H.haemorrhoicum	1	0	1	1	0	1	1	1	0	1	1	0	1	1	1	0	1	1	3	0	1	1	1	1	?	?	?
H. helvolum	1	0	0	1	0	1	1	0	1	1	1	0	1	1	?	?	0	0	0	0	1	0	1	1	1	?	1
H. hirsutum	1	1	1	2	1	1	1	3	0	1	2	0	1	1	0	0	0	0	2	0	1	1	1	1	1	0	1
H. maculipennis	1	0	2	1	0	1	1	2	0	1	3	0	1	1	0	2	1	0	1	0	1	1	1	1	0	0	0
H. pallipes	1	1	1	0	1	1	1	3	0	1	1	0	1	1	1	0	0	0	2	0	1	?	1	1	1	1	0
H. robustum	1	1	0	2	0	1	1	0	1	1	1	0	1	1	?	?	0	0	0	0	1	0	1	1	1	?	1
H. rufum	1	0	1	0	1	1	1	2	0	1	2	1	1	1	1	0	1	0	1	0	1	0	1	1	1	1	0

rescaled consistency index]. The tree searching commands (traditional search) used were: replications 1000, tbr, hold (max. trees) 10000, and the CI was used in the successive weighting with the macro "rewt.run".

In an attempt to quantify support for the clades on the tree, we have calculated the Bremer support (Bremer 1994) with TNT. We followed 2 procedures, one 'approximative' and the other 'exhaustive'. First, we calculated support values with TNT through the macro 'dobrem.run', although the macro file was modified in some commands at lines 72 (hold 20000) and 102 (hold 20 rep 100 tbr keep), with the strict consensus tree as the 'reference tree'. This first procedure provided approximate and fractioned (median) support values for the clades. Then, knowing the approximate maximum support value, we calculated Bremer support in TNT under an 'exhaustive' approach, with the following commands: hold 100000, hold/100, hold*, replications 1000 suboptimal 6, Bremer absolute supports. Progressively, we increased both the maximum number of trees in the memory (hold 300000) and the number of trees saved in each replication (hold/300) and decreased the suboptimal trees retained (suboptimal 5). We continued this procedure until the analysis reached stable support values with hold 600000, hold/600, hold*, replications 1000, suboptimal 4. Branch support was also verified by using Bootstrap (replications 1000, mult*100).

Geographical data (longitude/latitude) gathered for the species of *Heterostylum* are presented in the Supplementary Material (Appendix S1). These data were gathered from bibliographic references (Evenhuis & Greathead 1999; Hall & Evenhuis 1981; Painter et al. 1978) and also from specimens hosted in collections. The complete list of material examined is also presented as Supplementary Material (Appendix S2). For some species no precise locality data were available (e.g., "Mexico, Sinaloa" or "Canada, Alberta"), and for these localities we used geographical coordinates that are central to the area (e.g., for "Mexico, Sinaloa" the coordinates plot one point in the center of the state of Sinaloa).

In the cladistic biogeographical analysis, we used the classification scheme proposed by Morrone (2002), wherein the New World is divided into 3 regions: Nearctic, Neotropical and Andean, and Morrone (2001, 2004), and in which the Neotropical region is divided into 4 subregions (Caribbean, Amazon, Chacoan and Parana) and then into provinces. The following transitional zones are included: Mexican Transition Zone (between the Nearctic and Neotropical regions), and South American Transition Zone (between the Neotropical and Andean regions).

In the event-based biogeographical analysis, we carried out a Dispersal-Vicariance Analysis as implemented in the DIVA software (Ronquist 1996, 1997). DIVA analyses optimize and infer the distribution of the ancestral nodes based on current distributions of terminal taxa. We used the same spatial areas as above, but we did not include the transitional areas (Mexican Transition Zone/MTZ and South American Transition Zone/STZ). We tested different competing biogeographic scenarios by constraining the maximum number of areas (*maxareas*) allowed for the ancestral distribution. Therefore, we tested a more relaxed scenario with *maxareas* = 7 (which is the number of areas used) and then we defined a more restrict scenario with *maxareas* = 4.

Results and Discussion

Cladistics

From the data matrix (17 taxa × 27 characters) presented in Appendix 2, cladogram searches were carried out under the application of 2 different character weighting approaches: equal and successive weighting. The analysis using all characters, equally weighted, resulted in 6 most parsimonious cladograms with length = 68 steps, CI = 0.55, RI = 0.64. In the strict consensus tree (Fig. 1A), *Heterostylum* appears as monophyletic including 3 polytomic major clades, one formed by the Neotropical species: *H. haemorrhoicum* (*H. rufum* (*H. evenhuisi*, *H. maculipennis*)), the second also with Neotropical members (*H. ferrugineum* (*H. hirsutum*, *H. pallipes*)) and a third one, including all the Nearctic species, with no internal resolution.

After successive weighting was applied, a better resolved topology was obtained and the analysis yielded a single most parsimonious cladogram (Fig. 1B) with length = 68 steps, CI = 0.55, RI = 0.64. This cladogram is identical to one of the 6 cladograms resulting under equal weighting analysis.

The results (both equal and successive weighting analyses) support the monophyly of *Heterostylum* based on 4 unambiguous synapomorphies: row of setae on the ocellar tubercle separating the anterior ocellus from the others (character 1); row of setae on the anterodorsal surface of femur III (character 10, state 1); veins M1 and R5 merging near or at the point where R4 arises (character 13, state 1); and sperm pump longer than spermathecae (character 25, state 1); and one ambiguous synapomorphy: head as wide as or wider than thorax (character 7, state 1).

Based on the cladogram from the successive weighting analysis (Fig. 1B), 2 major groups within the genus were found: one large clade (Clade B) including the Neotropical species (*H. ferrugineum* (*H. hirsutum, H. pallipes*)) + (*H. haemorrhoicum* (*H. rufum* (*H. evenhuisi, H. maculipennis*))), the sister of a Nearctic clade (Clade A) with (*H. helvolum*, *H. robustum*) + *H. deani* + (*H. croceum*, *H. engelhardti*). With the acctran optimization, clade B appears supported by one synapomorphy, which is the presence of circular spots of different color on the tergites (character 19, state 1). However, this is misleading as, actually, only part of clade D presents this state. Therefore, the following corrected scenario for character 19 (pattern of coloration on abdominal tergites) in clade B is: presence of a central longitudinal stripe (state 2) ambiguously support the clade C (*H. ferrugineum* (*H. hirsutum, H. pallipes*)), while in clade D the loss of any mark (state 3) is observed in *H. haemorrhoicum*, and circular spots are present in the clade (*H. rufum* (*H. evenhuisi, H. maculipennis*))

This last clade is supported by one unambiguous synapomorphy, the presence of golden hairs on the mesonotum and dark brown hairs on the pleura (character 8, state 2).

Although no higher-level phylogeny that includes *Heterostylum*, *Triploechus* and *Apiformyia* together has been published to date, some authors have suggested and discussed their morphological affinities (Hall 1976; Hall & Evenhuis 1981; Yeates 2008). These affinities have been based mainly on the sinuous posterior eye margin, which is uncommon in Bombyliinae. Also, among the 3 genera, *Apiformyia* could possibly be recovered closer to the root in the phylogeny, because its female terminalia lacks the sand chamber and acanthophorite spines. In the present study, the outgroup was composed by *A. australis* and 3 species of *Triploechus*: 2 Andean (*T. bellus* and *T. heteronevrus*) and one Nearctic (*T. novus*), besides *Toxophora aurea*, which was used to root the resulting trees. We did not include all 5 species of *Triploechus* in the analysis, but its monophyly was recovered using the 3 representative species. Furthermore, these preliminary data indicated a sister-group relationship between *Triploechus* and *Heterostylum*.

Biogeography

The origin of the Bombyliidae (in a broader sense, including Mythicomyiinae, as recently supported by Trautwein et al. 2010) was hypothesized to have taken place in the Middle Jurassic, based on congruence among biogeographical, molecular and fossil data (see Lamas & Nihei 2007), whereas the diversification of the Bombyliinae lineage has occurred since the Early Cretaceous (125 Mya), according to molecular dating (Wiegmann et al. 2003). Fossil records for Bombyliinae and Bombyliini, where *Heterostylum*, *Triploechus*, and *Apiformyia* have been allocated, are rare, and this scarcity has hampered to further discussions about the ancient diversification of these taxa and also of any other bombyliine group. For example, the oldest fossil records for Bombyliini are described from the Eocene epoch (Evenhuis 1997; Lamas & Nihei 2007). These fossil records are represented by *Bombylius* from Germany (Miocene), France (Oligocene), and the Baltic region (Eocene/Oligocene); and *Dischistus* from France (Oligocene). However, given its fragmentary nature, a literal reading of the fossil record should be regarded as misleading (Heads 2005), and although not yet found, it is still possible that pre-Cretaceous fossils of the Bombyliidae lineage will be discovered.

Based on the cladogram of Fig. 1B, the area cladogram derived for *Heterostylum* provides evidence for a spatial disjunction between the Nearctic and Neotropical clades (clades A and B) associated with the Mexican Transition Zone (MTZ,
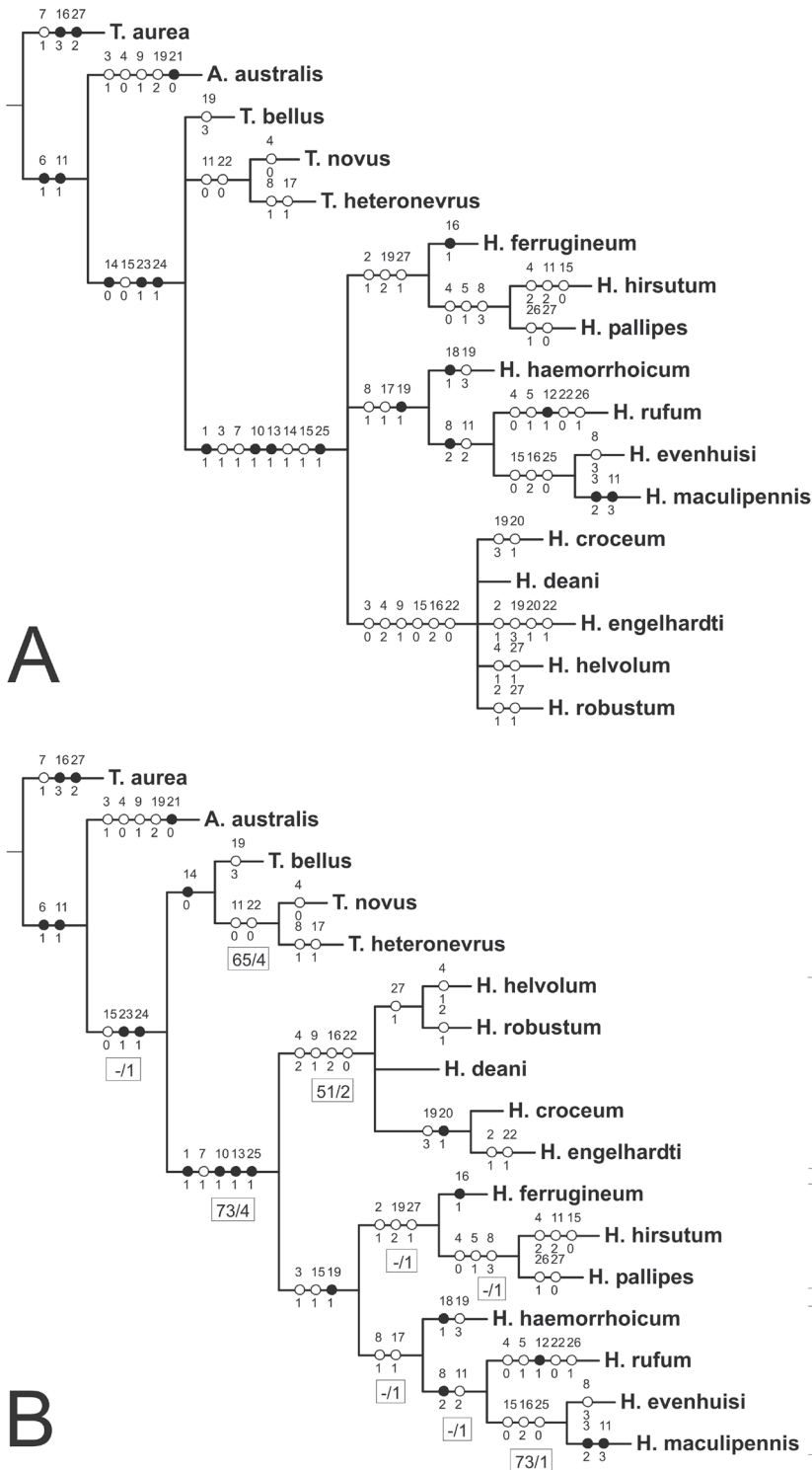
Fig. 1. A: Consensus tree of 6 most parsimonious trees resulting from equal weighting analysis, under *acctran* optimization (length = 74 steps, CI = 0.51, RI = 0.57). B: Single most parsimonious tree resulting from successive weighting analysis, under *acctran* optimization (length = 68 steps, CI = 0.55, RI = 0.64). Black circles represent unique changes; white circles represent multiple changes. Bootstrap values and Bremer support values are shown inside rectangles below branches, separated by "/".

see Morrone 2004, 2006). The Nearctic clade ranges widely over the western and central USA and northern and central Mexico, with the MTZ as the southern boundary (Figs. 2 and 3). The Neotropical clade B extends throughout the Neotropical region (Figs. 2 and 3). It is interesting to note that the genus is absent towards northeastern USA and the south of Brazil and Paraguay, as well as in eastern North America and west of the Andes. On the other hand, some of the lacunae in the distributional pattern are probably due to deficient sampling efforts, as might be the case in Colombia, Guyana, Bolivia, Peru, and southern continental Central America.

Although the origin of *Heterostylum* and the early diversification of Bombyliini lineages are not elucidated, a separate examination of the phylogenetic and distributional pattern of *Heterostylum* can add valuable data about the spatial history of the tribe. The distributional pattern of *Heterostylum* shows a generic distribution congruent with the phylogenetic pattern, as the basalmost dichotomy diverging to Nearctic and Neotropical clades (Fig. 1B) is congruent with the distributional disjunction between the Nearctic and Neotropical clades (Figs. 2 and 3). Although in close geographical proximity, the Nearctic and Neotropical clades do not overlap each other. The disjunction of *Heterostylum* is spatially coincident with the boundaries of MTZ. The biogeographical scenario presented in Fig. 3 is suggested based upon the phylogenetic hypothesis proposed here (Fig. 1B). Yet, in our analysis the monophyly of clade B had no Bremer support, whereas clade A had bootstrap (51%) and Bremer supports (2). Furthermore, the 'Nearctic' definition is used here in the broad sense, as the Nearctic clade extends over areas within the Nearctic region and also southward through the MTZ (see Morrone 2004, 2006).

A number of biogeographers have been focusing their studies on the MTZ, correlating and explaining its biota with both Nearctic and Neotropical influences. Croizat (1958) suggested a node in southern Mexico as a major node regarding patterns on the intercontinental scales. Halffter (1964, 1987, and others) pioneered biogeographical studies on insects in the MTZ. In fact, Gonzalo Halffter was the most experienced authority on the MTZ history and biogeography. He regarded the complexity of MTZ as potentially responsible for the scarcity of biogeographical studies in that area (Halffter 1964). The Nearctic range of *Heterostylum* is congruent with the insect distributional patterns pointed out by Halffter (1987), wherein the species occurring north of the Transmexican Volcanic Belt have more affinities with the Nearctic fauna than with the Neotropical one.

What geological or biogeographical event could be associated with the basal divergence of *Heterostylum* (Figs 2 and 3)? The Transmexican Volcanic Belt has a recent origin, since its formation began during the Oligocene (Halffter 1987). Perhaps another ancient event in the Caribbean (and related to the formation of the Caribbean Plate) could be involved. Alternatively to the traditional north-south Oligocene division across the Transmexican Volcanic Belt, Escalante et al. (2007) suggested the occurrence of an earlier Caribbean event during the Paleocene dividing the Mexican biota through an east-west division. These authors suggested a biogeographical relationship of eastern Mexico with the early supercontinent Gondwana, while its relationship with the adjacent areas (Central America and Caribbean) was not precisely discussed. The geological event argued as responsible for generating that pattern, the eastward movement of the Caribbean Plate, took place in the Paleocene (Escalante et al. 2007) and was related to the formation of the Proto-Greater Antilles during the Late Cretaceous. Nevertheless, we regard as equally valid the association of the east-west division of Mesoamerica with the early formation of the Proto-Greater Antilles. The Cretaceous Volcanic Arc formed during the Late Cretaceous and was deformed dynamically across the Paleogene while firmly accompanying the eastward movement of the Caribbean plate (Iturralde-Vinent & MacPhee 1999). The age of this geological event in dating the basal divergence of *Heterostylum* is congruent with the molecular dating of the subfamily Bombyliinae inferred by Wiegmann et al. (2003) as the Early Cretaceous (125 Mya).

In this context, the early formation of the Caribbean islands, derived from the Cretaceous-Paleogene Volcanic Arc, could explain the current distribution pattern of *Heterostylum* species throughout the Mesoamerican areas, with regard to both presence and absence of species. *Heterostylum* is highly diversified through the Caribbean islands, nearly absent on the Central American continental landmass, and reasonably diversified in the southern Nearctic (Mexico), but with no overlap between the Caribbean/Central American and the Nearctic species. The continuous eastward movement of the Cretaceous-Paleogene Volcanic Arc, since its formation in the Late Cretaceous and its dynamic conformation during the Paleogene until the current Caribbean was consolidated, paralleled the eastward movement of the Centroamerican Arc, a neighboring western plate bearing the early landmasses that formed the present continental region of Central America (Iturralde-Vinent & MacPhee 1999).

The basal diversification of the Neotropical clades C and D indicates a distributional fragmentation from the Caribbean areas (Figs. 4 and 5, see also Fig. 2). All species in clade C have a Caribbean distribution, and although most of the species have a widespread distribution in South American semiarid areas, this clade has not di-
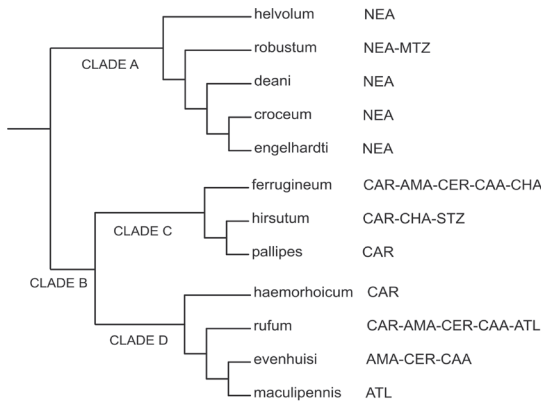
Fig. 2. Area cladogram for *Heterostylum* (Legends: NEA, Southern Nearctic; AND, Andean region; CAR, Caribbean subregion; AMA, Amazonian subregion; CER, Cerrado; CAA, Caatinga; ATL, Atlantic Forest (=Paraná subregion); CHA, Chaco; STZ, South American Transition Zone; MTZ, Mexican Transition Zone).

versified outside the Caribbean (Fig. 2), i.e., there is no endemic species outside the Caribbean. With regard to clade D, the 2 species closest to the basal node (*H. haemorrhoicum* and *H. rufum*) occur in the Caribbean (Figs. 2 and 5). Similarly to some species in clade C, *H. rufum* occurs in the Caribbean but is also widespread in South America, ranging through the Amazonia, Cerrado, Caatinga, to the Atlantic domains. However, unlike clade C, this other Neotropical clade is distinctive in having species occurring in the Atlantic Forest (Fig. 2). Both *H. rufum* and *H. maculipennis* occur in the Atlantic Forest, although only the latter actually occurs in humid environments of the Atlantic Forest (see discussion below). Again, unlike clade C, this clade has diversified into South America, with 3 endemic species in Brazil.

Alternative Biogeographic Scenarios

Besides the taxon-area cladograms (Fig. 2), we performed a DIVA analysis to test and refine the results obtained and discussed above. DIVA is an event-based analysis and can recognize biogeographical processes (vicariance, dispersal and dispersion) which possibly originated the current taxon distribution. The DIVA analysis performed using the phylogenetic hypothesis and distributional data of *Heterostylum* produced a similar interpretation but it also derived alternative scenarios (Figs. 6A-D), which will be discussed herein. In a relaxed analysis, not constraining the maximum number of areas of the ancestral distribution (*maxareas* = 7) (see methodology description above), DIVA resulted in a biogeographical scenario (Scenario 1, Fig. 6A) with ancient origin and diversification, wherein the ancestral

distribution is inferred to be wide (= sum of all areas, ABCDEFG) with the occurrence of subsequent vicariant events. Sympatric distributions (overlapping) between terminal taxa and between clades were resolved by assuming at least 10 ad hoc events, however, the DIVA algorithm cannot correctly distinguish whether dispersion or dispersal occurred, i.e., simple range expansion or a true dispersal process. This correction can be made 'manually', post-algorithm. Moreover, DIVA may often produce some unrealistic optimizations for some nodes, as seen in Scenario 1 (Fig. 6A), when inferring the ancestral distribution BG (Caribbean + Atlantic Forest) for the node of terminals 9 to 12. This disjunct ancestral distribution is spurious. DIVA has some pitfalls and inherent problems, and many of them were raised and well discussed in Kondandaramaiah (2010). For this reason, one should be very careful when carrying out a DIVA analysis, especially in interpreting its results (the optimized scenarios).

Alternative (and more realistic) scenarios were optimized constraining *maxareas* on DIVA, as in Figs. 6B and 6C. Both scenarios applied *maxareas* = 4, and these 2 scenarios differ only in the ancestral distribution assumed for the basalmost node: ABC (Nearctic + Caribbean + Amazon) in Scenario 2, and AB (Nearctic + Caribbean) in Scenario 3. The former (Fig. 6B) proposes an ancient vicariant hypothesis for *Heterostylum* as in Scenario 1, although not completely widespread. In contrast, Scenario 3 proposes a northern origin for *Heterostylum* with a north-to-south dispersalist hypothesis. Both scenarios assume widespread distribution of the terminals by models involving events of dispersion (e.g., terminals 10 and 11 in Scenario 2) and dispersal (e.g., node of clade D in Scenario 2).

Interestingly, DIVA analyses did not infer any scenario with the basalmost distribution without including the Nearctic region. Meanwhile, another serious inherent problem is that DIVA is not able to infer any event in the basalmost node other than vicariance. This is to say that at this node, dispersion or dispersal events will never be inferred by DIVA analyses. (For that, there should be the addition of taxa intermittently joined to the root.) Hence, to regard such events inherently ignored by DIVA, we can manually include additional ad hoc events to the basalmost node, as in Scenario 4, where the ancestral distribution ABC would have been reached after a dispersal event (+A) with subsequent divergence into A and BC. Observe that this is the same as presented in Scenario 2, but contrasting in the explanation for the ancient distribution and the associated event (vicariance in Fig. 6B, dispersal in Fig. 6D). As a consequence, while Scenario 2 supports an ancient vicariant scenario with a widespread ancestral distribution, Scenario 4 supports a southern origin with a south-to-north dispersalist scenario.

Fig. 3. Geographical distribution of the genus *Heterostylum*; with the disjunctional basal dichotomy separating the Nearctic and Neotropical clades (clades A and B, respectively).

Did *Heterostylum* Species Diversify Mostly within Forested Areas?

The species distributions maps (Figs. 7 and 8) and the area cladogram (Fig. 2) provide interesting information about species distributions and endemicity. It is well known that bombyliids often inhabit drier environments, although this would not seem to be the case for *Heterostylum* species by a mere glance at the maps. Furthermore, it is quite curious that no endemic species occur in the semi-arid areas of South America east of the Andes, namely the Cerrado, Caatinga, and Chaco (although *H. duocolor* is known only from the type-locality in the Chaco). However, these superficial impressions are misleading.
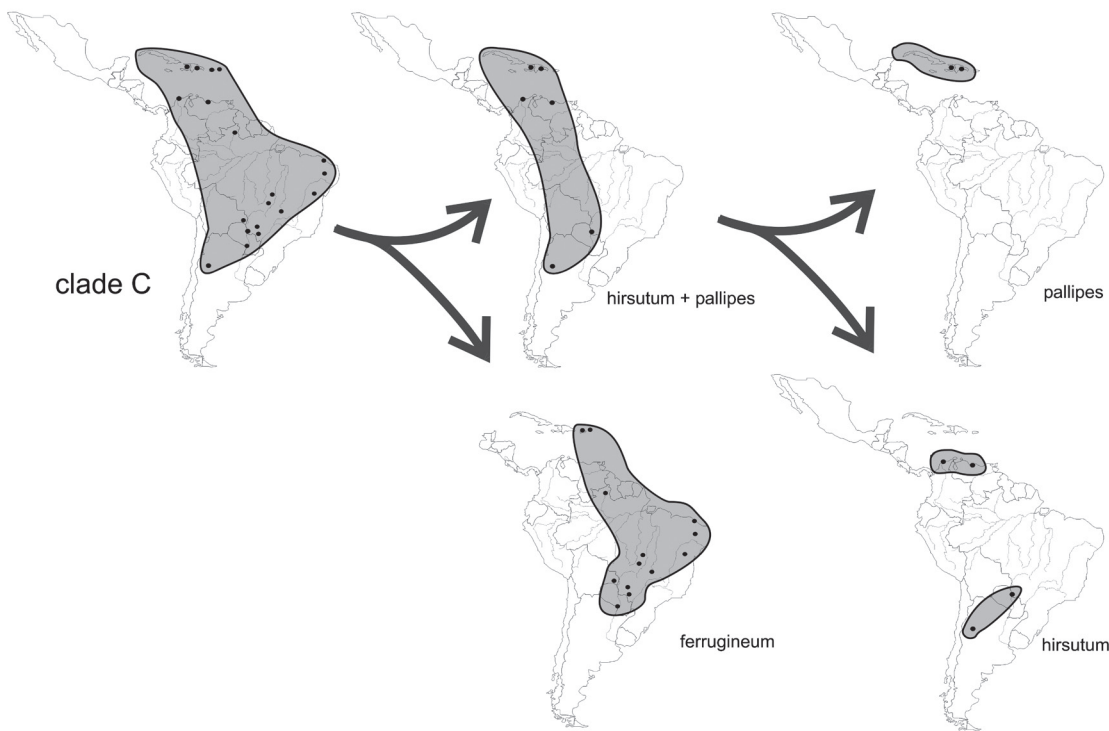
Fig. 4. Sequence of diversification of the Neotropical clade C.
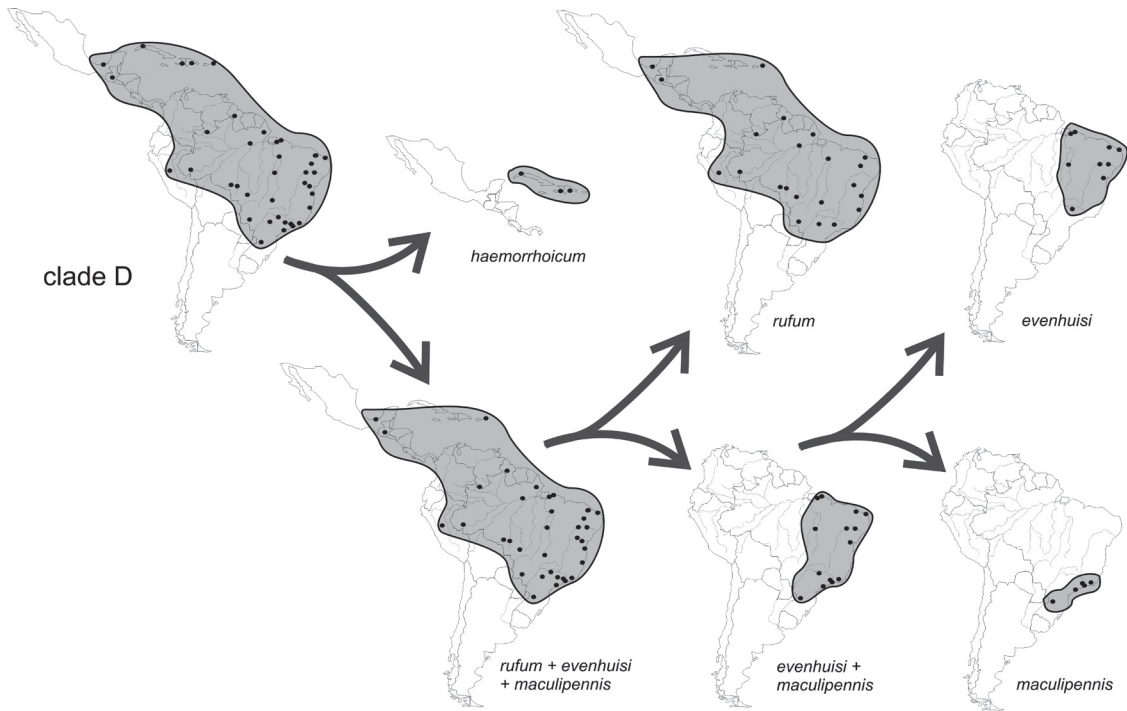
Fig. 5. Sequence of diversification of the Neotropical clade D.
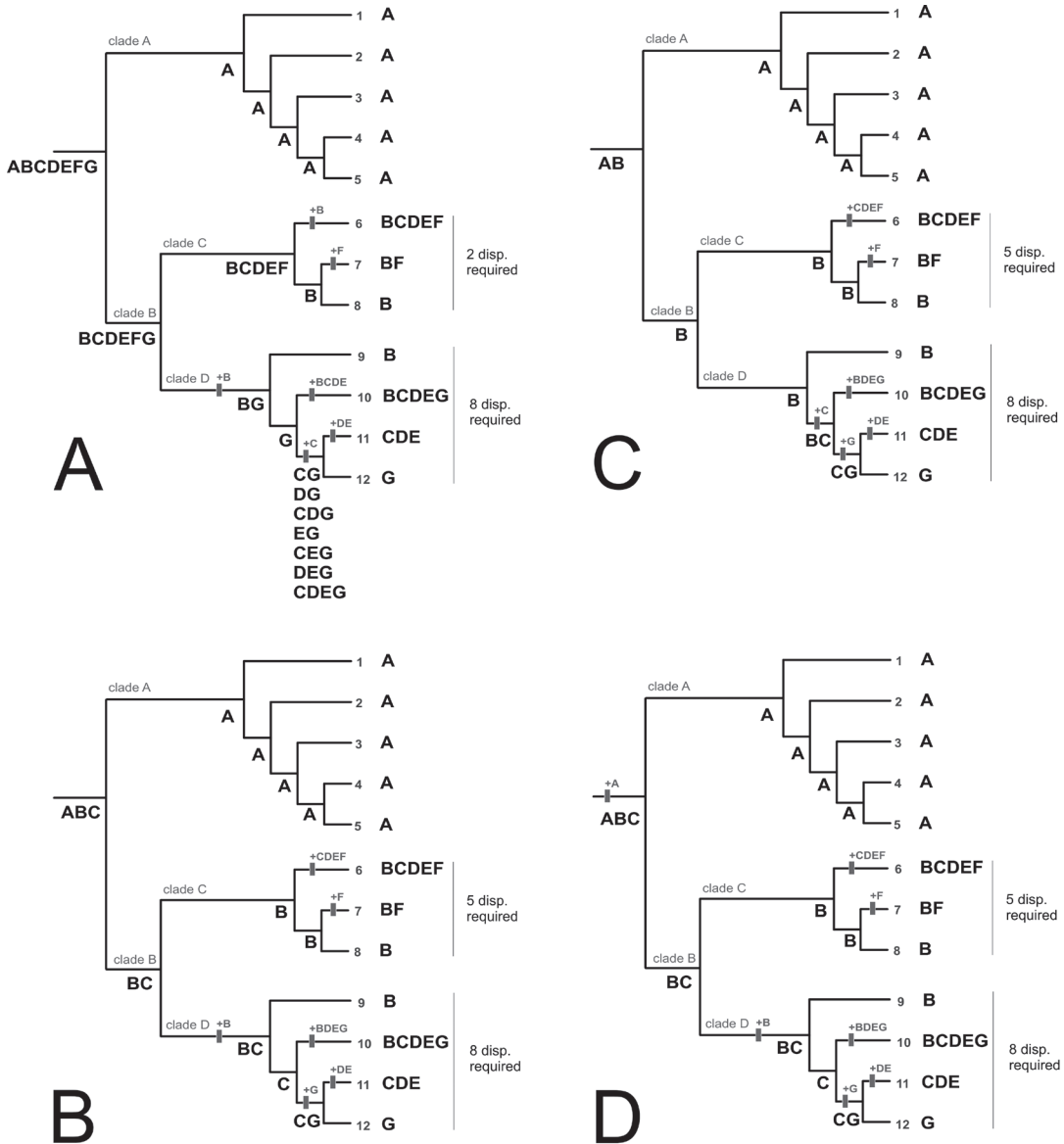
Fig. 6. Event-based analysis by DIVA. A: *Scenario 1 (ancient widespread origin)*: biogeographic scenario using *maxareas* = 7, required 10 events of dispersion/dispersal. For the node 11+12 we arbitrarily chose the ancestral distribution CG (Amazon + Atlantic Forest) within the 7 options resulting for the node. B: *Scenario 2 (ancient restrict origin)*: biogeographic scenario using *maxareas* = 4, required 13 events of dispersion/dispersal. The basalmost ancestral distribution occurring in ABC (Nearctic + Caribbean + Amazon). C: *Scenario 3 (northern origin)*: same optimization as Scenario 2, but differing in the basalmost ancestral distribution restricted to AB (Nearctic + Caribbean). D: *Scenario 4 (southern origin)*: same optimization as Scenario 2, but including an additional event in the cladogram root unlikely to be inferred by DIVA: the dispersal of the ancestral occurring from BC to A, then becoming ABC. Total events required is 14. (Legends: numbers refers to species of *Heterostylum*; letters refers to areas: A, Nearctic; B, Caribbean; C, Amazon forest; D, Cerrado; E, Caatinga; F, Chado; G, Atlantic forest. For Scenarios 2, 3 and 4, DIVA analyses optimized alternative distributions for some nodes; please see the Supplementary Material)

Heterostylum rufum is widespread in the Caribbean and Amazonian subregions, and Cerrado, Caatinga and Atlantic Forest (Fig. 7).

Heterostylum rufum certainly occurs in the Amazonian subregion, but in the Amazon Forest this appearance is artifactual for some localities (see
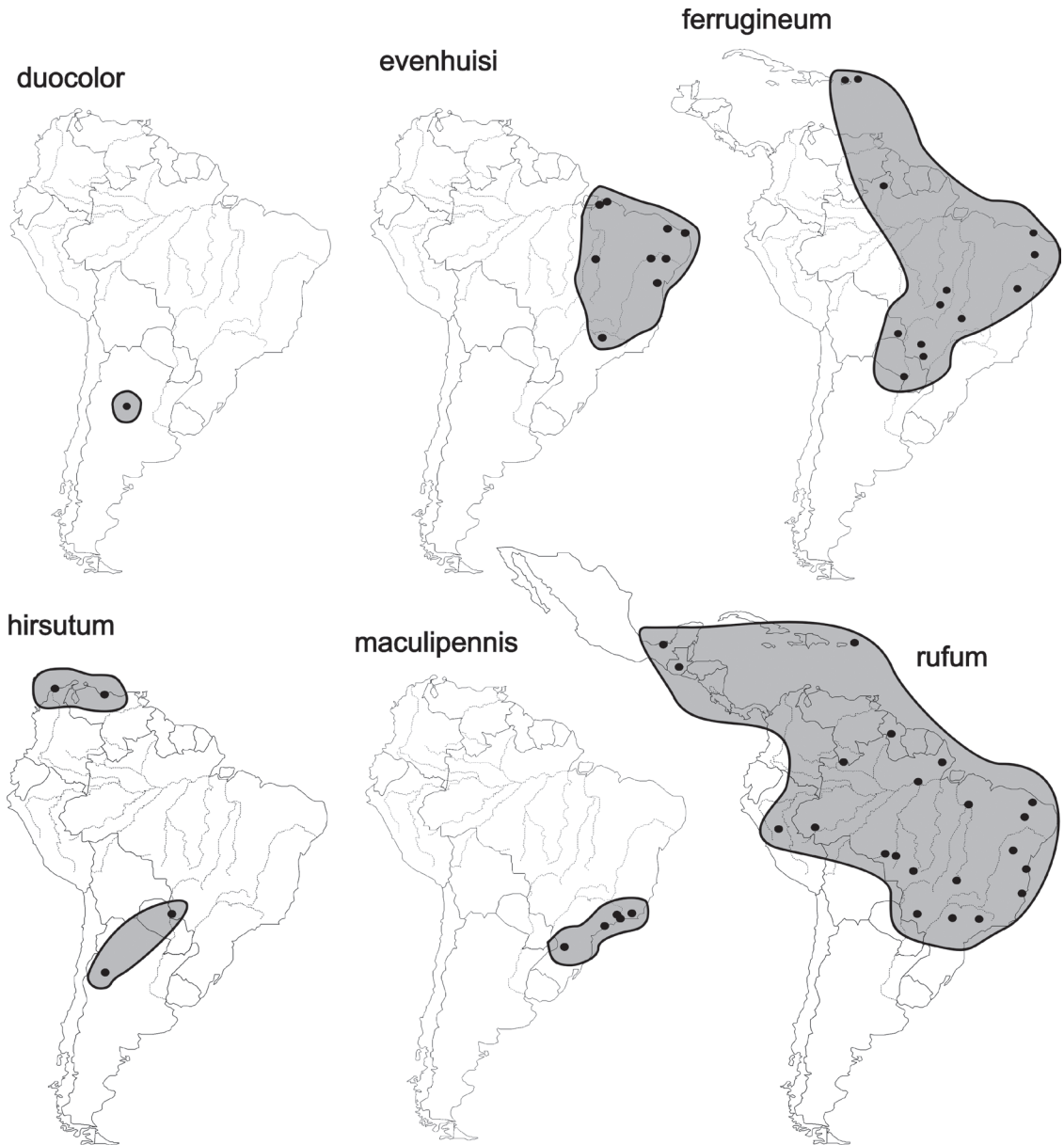
Fig. 7. Geographical distribution of *Heterostylum* species: *H. duocolor, H. evenhuisi, H. ferrugineum, H. hirsutum, H. maculipennis*, and *H. rufum*.

Appendices 3 and 4). Some of the recorded localities within the Amazonian subregion are the following: 1) Serra Norte (Pará), in southeastern Pará, is known to have "campos rupestres" vegetation (a kind of Cerrado); 2) Alter do Chão (Pará, Rio Tapajós) has sandy beaches along the Tapajós River; 3) Imperatriz (Maranhão) has "mata dos cocais" vegetation, a transitional ecosystem between the Amazon Forest and the semi-arid

Caatinga in northeastern Brazil; and 4) Porto Platon (Amapá) is covered by Cerrado vegetation. Similarly, *H. rufum* occurs in the Atlantic Forest (=Paraná subregion) but, in this case, in both humid and semi-arid environments: 1) the locality Sertãozinho (São Paulo) is covered mainly by Cerrado; 2) Conceição da Barra (Espírito Santo) is a coastal locality covered by "restinga", a semi-arid vegetation bordering beaches; and
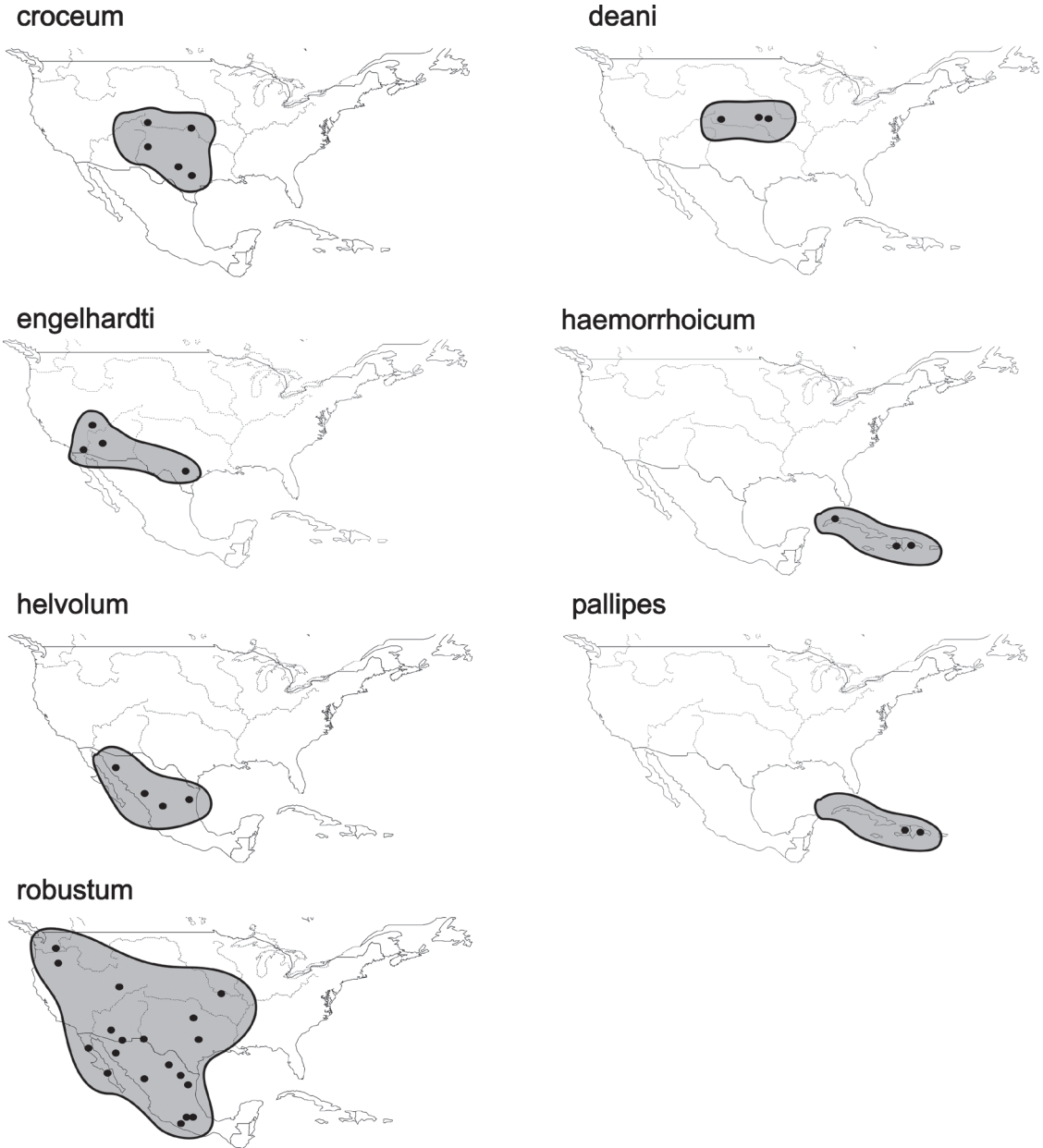
croceum

deani

engelhardti

haemorrhoicum

helvolum

pallipes

robustum

Fig. 8. Geographical distribution of *Heterostylum* species: *H. croceum, H. deani, H. engelhardtii, H. haemorrhoicum, H. helvolum, H. pallipes* and *H. robustum*.

3) Marília (São Paulo) has semi-deciduous forest vegetation.

The occurrence of *H. ferrugineum* in the Amazonian subregion is based on a record from Boa Vista (Roraima), which is covered by Cerrado vegetation. The remaining records are in the Caribbean and in the semi-arid diagonal (Cerrado + Caatinga + Chaco). In the Caatinga, the species is widespread, reaching the northeastern Brazilian

coast (Ceará) and also extending up to the transition between the Caatinga and Atlantic Forest (Santa Rita-Bahia). Its absence from the Amazon Forest is conspicuous, such as in the sister-clade *H. hirsutum* + *H. pallipes*.

The records known for *H. hirsutum* (Fig. 7) are located separately in 2 disjunct areas (as far as we know it): one north, on the coastal regions of Venezuela and Colombia, and another south,

on northwestern Argentinean Andes and south-western Brazilian Chacoan area. Besides these confirmed records, the type-locality is mentioned only as Brazil (Cunha et al. 2007). It is also recorded in Paraguay, but unfortunately, this occurrence was not confirmed during our search (by examination of collections and literature review). Based on the reliable records, the known and confirmed distribution of this species is restricted to the Caribbean subregion, Chacoan subregion, and the South American Transition Zone (STZ).

Only one species of *Heterostylum*, *H. duocolor*, occurs exclusively in the Chacoan subregion, with a single locality so far recorded in the Argentinean Patagonia (Córdoba). This species was not included in the cladistic analysis because of the unavailability of material for study (the type is lost, and it has not been recognized by subsequent authors).

On the other hand, *H. maculipennis* is actually endemic to humid environments of the Atlantic Forest (e.g., the localities of Nova Friburgo and Itatiaia in the state of Rio de Janeiro, and Nova Teutônia in Santa Catarina), although it also occurs in coastal areas with comparatively dry vegetation (e.g., the localities of Praia Grande, state of São Paulo, and Angra dos Reis, state of Rio de Janeiro).

CONCLUSIONS

The hypothesized phylogenetic pattern for *Heterostylum* separates the species into 2 clades, a Nearctic and a Neotropical, with 5 and 7 species, respectively. The phylogenetic pattern is congruent with the distributional pattern as the basalmost dichotomy between the Nearctic and Neotropical clades is congruent with the spatial disjunction between both clades. The disjunction of *Heterostylum* is spatially coincident with the Mexican Transition Zone.

The biogeographical analysis presented and discussed above supports a Caribbean geological event (e.g., the formation and history of the Proto-Greater Antilles, and the eastward movement of the Caribbean Plate) as responsible for the ancient diversification of major lineages of *Heterostylum*. The absence of *Heterostylum* species from the Central American continental landmass, in contrast to the high diversity found in the Caribbean areas (5 species), reinforces the role of the Cretaceous-Paleogene Volcanic Arc for the diversification of the genus. It is quite contrasting how vicariance can be consistently postulated to explain the distributional pattern of the Neotropical species, whereas the Nearctic species overlap broadly in their distributions and are hence difficult to explain. On the other hand, the event-based biogeographical analysis (DIVA) recognized events, which supported the same scenario discussed above, but also supported and indicated alternative scenarios for *Heterostylum*. However, unless we know the resolution of other Bombyliini closely related with *Heterostylum*, and reliable fossil record and dating of the lineages, the biogeographical history of the group will remain unclear.

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