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Evolution of *Trichobaris* (Curculionidae) in relation to host plants: Geometric morphometrics, phylogeny and phylogeography

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ABSTRACT

The family Curculionidae (Coleoptera), the "true" weevils, have diversified tightly linked to the evolution of flowering plants. Here, we aim to assess diversification at a lower taxonomic level. We analyze the evolution of the genus *Trichobaris* in association with their host plants. *Trichobaris* comprises eight to thirteen species; their larvae feed inside the fruits of *Datura* spp. or inside the stem of wild and cultivated species of Solanaceae, such as potato, tobacco and tomato. We ask the following questions: (1) does the rostrum of *Trichobaris* species evolve according to the plant tissue used to oviposit, i.e., shorter rostrum to dig in stems and longer to dig in fruits? and (2) does *Trichobaris* diversify mainly in relation to the use of *Datura* species? For the first question, we estimated the phylogeny of *Trichobaris* based on four gene sequences (nuclear 18S and 28S rRNA genes and mitochondrial 16S rRNA and COI genes). Then, we carried out morphogeometric analyses of the *Trichobaris* species using 75 landmarks. For the second question, we calibrated a COI haplotype phylogeny using a constant rate of divergence to infer the diversification time of *Trichobaris* species, and we traced the host plant species on the haplotype network. We performed an ancestral state reconstruction analysis to infer recent colonization events and conserved associations with host plant species. We found that ancestral species in the *Trichobaris* phylogeny use the stem of *Solanum* plants for oviposition and display weak sexual dimorphism of rostrum size, whereas other, more recent species of *Trichobaris* display sexual dimorphism in rostrum size and use the fruits of *Datura* species, and a possible reversion to use the stem of Solanaceae was detected in one *Trichobaris* species. The use of *Datura* species by *Trichobaris* species is widely distributed on haplotype networks and restricted to *Trichobaris* species that originated ca. 5 ± 1.5 Ma. Given that the origin of *Trichobaris* is estimated to be ca. 6 ± 1.5 Ma, it is likely that *Datura* has played a role in its diversification.

1. Introduction

It is rare to see the process of speciation in nature. The use of both phylogenetic and phylogeographic approaches can help in understanding the evolution of species. Ideally, one should sample all species of a taxonomic group, such as a genus, and test that all named species constitute evolutionary entities within that group to rule out the effects of other processes, as well develop explanations for observed patterns (Avise and Johns, 1999; Holt and Jønsson, 2014). The phylogenetic hypothesis provides an indirect record of the sequence of lineage-divergence events (Barraclough and Nee, 2001) that can be complemented with phylogeographic analysis. This permits the recognition of the geographic and spatial genetic structure of populations within a species at early stages of differentiation (Avise, 2000; Gavrilets, 2003). These integrative studies can provide insights into how speciation proceeds (e.g., Chen et al., 2017; Supple et al., 2014).

Speciation in phytophagous insects has long been associated with plant diversification (Ehrlich and Raven, 1964; Farrell, 1998). The majority of phytophagous species in the order Coleoptera belong to the superfamilies Chrysomeloidea and Curculionoidea (Grimaldi and Engel, 2005). Curculionidae beetles, known as weevils, include approximately 62 000 described species (Oberprieler et al., 2007). Weevil diversity is associated mainly with angiosperms (McKenna et al., 2009) due to their ability to exploit different plant tissues (Marvaldi et al., 2002). However, weevil diversity has been associated with presumed key innovations, such as the rostrum's length and shape, endophagous larvae and

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Table 1
Sampling sites of *Trichobaris* weevils on their host plant species in Mexico and USA. (*) COI gene sequence from previous publications (De-la-Mora et al., 2015; in press).

Number	Host plant	State	Country	Sampling site	Coordinates	# insects DNA data	# insects Morphology data
1	*D. discolor*	Baja California	Mexico	129	26°0′21.46″N, 111°20′35.34″W	5	7
2	*D. inoxia*	Hidalgo	Mexico	505	20°34′54.50″N, 99°33′50.58″W	5	2
3	*D. inoxia*	Oaxaca	Mexico	516	16°28′46.10″N, 96°13′3.51″W	1	5
4	*D. pruinosa*	Oaxaca	Mexico	415	16°40′2.60″N, 96°22′48.98″W	1	___
5	*D. quercifolia*	Hidalgo	Mexico	207	20°19′3.79″N, 99°9′34.98″W	20	12
6	*D. stramonium*	Puebla	Mexico	119	19°5′38.49″N, 98°24′34.89″W	6	___
7	*D. stramonium*	Oaxaca	Mexico	104	17°14′11″N, 96°25′53.77″W	9	___
8	*D. stramonium*	Hidalgo	Mexico	106	20°8′18.98″N, 98°55′21.38″W	5	___
9	*D. stramonium*	Puebla	Mexico	110	18°54′8.23″N, 98°26′21.15″W	19	___
10	*D. stramonium*	Puebla	Mexico	109	18°56′27.15″N, 98°6′53.46″W	18	24
11	*D. stramonium*	Oaxaca	Mexico	113	16°55′11.92″N, 96°23′6.10″W	20	1
12	*Physallis* sp.	Hidalgo	Mexico	T18	20°35′23.59′N, 99°37′11.22″W	2	2
13	*Solanum eleagnifolium*	Tamaulipas	Mexico	722	25°58′45.05″N, 98°5′45.66″W	1	___
14	*Solanum rostratum*	SanLuisPotosí	Mexico	620	23°33′36.99″N, 100°37′41.69″W	8	1
15	*D. wrightii*	California	USA	5SD	33°46′4.14″N, 116°19′28.23″W	1	3
16	*D. wrightii*	California	USA	8SD	___	3	___
17	*D. wrightii*	California	USA	20SD	33°59′46.45″N, 116°34′43.69″W	1	___
18	*D. wrightii*	Arizona	USA	0Az	32°35′32.79″N, 110°50′56.54″W	3	1
19	*D. wrightii*	Arizona	USA	1Az	32°36′50.10″N, 110°49′57.91″W	3	___
20	*D. wrightii*	Arizona	USA	2Az	32°58′42.19″N, 110°46′8.08″W	3	3
21	*D. stramonium*	Arizona	USA	4Az	32°3′48.57″N, 110°17′3.42″W	1	1
22	*S. eleagnifolium*	Arizona	USA	5Az	32°1′54.69″N, 110°18′40.51″W	2	___
23	*D. wrightii*	Arizona	USA	6Az	33°5′25.83″N, 112°2′1.26″W	3	2
24	*S. eleagnifolium*	Texas	USA	Tx1	33°52′55.49″N, 98°28′53.04″W	2	1
25	*S. eleagnifolium*	Texas	USA	Tx2	32°54′27.50″N, 97°34′50.64″W	1	___
26	*S. carolinense*	Virginia	USA	4VA	37°37′22.4″N, 77°59′13.1″W	4	___
27	*S. tuberosum*	Virginia	USA	4VA	37°37′11.4″N, 77°59′18.8″W	2	___
28	*D. stramonium*	Virginia	USA	5VA	37°30′38.5″N, 77°42′12.9″W	2	4
29	*S. carolinense*	Virginia	USA	1VA	37°39′49.8″N, 77°53′30.1″W	1	___
30	*S. carolinense*	Virginia	USA	2VA	37°32′28.6″N, 77°53′36.8″W	4	___
31	*S. carolinense*	Virginia	USA	3VA	37°32′56.3″N, 77°55′01.8″W	5	___
32	*D. stramonium*	Virginia	USA	3VA	37°31′31.5″N, 77°52′11.5″W	2	___
33	*D. ceratocaula*	Durango	Mexico	Map	25°50′1.35″N, 103°50′55.46″W	___	5
34	*D. stramonium*	Michoacán	Mexico	102	19°40′46.19″N, 101°15′12.68″W	___	3
35	*Datura* sp.	Baja California	Mexico	Uru	31°34′03.2″N, 116°25′19.6″W	___	6
36	*Datura* sp.	Baja California	Mexico	SAN	32°06′55.8″N, 166°30′02.8″W	___	7
37	*Solanum* sp.	Michoacán	Mexico	630	19°41′9.87″N, 101°13′46.56″W	___	3
38	*D. quercifolia*	Guanajuato	Mexico	230	20°25′20.6″N, 99°58′37.9″W	___	18
39	*D. stramonium*	Edo. de Mexico	Mexico	Teo	19°40′48.75″N, 98°52′26.51″W	*	2
40	*D. stramonium*	Guerrero	Mexico	RC	17°32′27.34 N, 99°28′19.27″W	*	1
41	*D. discolor*	Oaxaca	Mexico	314	16°47′11.61″N, 96°12′42.34″W	*	14
42	*D. discolor*	BajaCaliforniaN.	Mexico	828	32°11′28.14″N, 114°55′19.63″W	*	7
43	*D. wrightii*	California	USA	16SD	34°11′19.90″N, 116°26′4.89″W	*	2
44	*D. wrightii*	California	USA	18SD	34°14′9.57″N, 116°26′23.43″W	*	2
					Total	163	139

geniculate antennae and, in some groups, their association with fungi (Oberprieler et al., 2007; Matsubayashi et al., 2010).

Weevils are pests of many economically important plants, such as chestnut, sugarcane, banana, cotton, raspberry, avocado, mango, pecan, pepper, alfalfa and sunflower (Avtzis et al., 2013; Kuroki and Kodama, 1987; Lemic et al., 2016; Shankar et al., 2015; Barr et al., 2013; Parra et al., 2009; Castañeda-Vildozola et al., 2015; Bierig, 1939; Basio et al., 1994, Mynhardt, 2006; Capinera, 2005; Iwase et al., 2015; Charlet, 1983). The historical relationship between weevils and their host plants has scarcely been explored at the macroevolutionary level. Determination of ancestral and recently colonized host plant species is also a topic that remains understudied (e.g., Iwase et al., 2015; Kuester et al., 2012). Some studies have shown the ecological and evolutionary consequences of host shifts for biological control and to sustain productivity (Oliveira et al., 2012).

At a microevolutionary level, the effect of the host plant species on the genetic variation of weevil species in a genus is, likewise, a poorly explored question (e.g., Hernández-Vera et al., 2010; Kohyama et al., 2014). In some instances, no clear relationship between the genetic variation of weevils and their host plants has been found. In the stenophagous capitulum weevil (*Larinus cynarae*), the primary distribution of its genetic diversity indicates a geographic division followed by branching of *L. cynarae*'s lineage into different host plants (Briese et al., 1996). However, in the boll weevil, *Anthonomus grandis*, three morphological forms associated with species of *Gossypium* have been described; although some haplotypes appeared related to either wild or cultivated cotton (Barr et al., 2013), most genetic differentiation is due to their geographical distribution (Kuester et al., 2012). In an extreme case, genetic variation of alfalfa weevil, *Hypera postica*, is not associated with its host plant or with its geographic distribution (Iwase et al., 2015). However, the interaction between weevils and their host plants can result in a clear association of genetic variation of both organisms, as exemplified by *Curculio hilgendorfi* and *Castanopsis sieboldi* (Aoki et al., 2011), or as a shared phylogeographical pattern (Aoki et al., 2009).

Weevil species of the genus *Trichobaris* (Curculionidae: Baridinae) use plants in the family Solanaceae as food and for larval development inside stems or fruits. The female digs a hole in the stem or fruit to oviposit; hatching larvae feed inside until pupation; and once metamorphosis occurs, adults emerge to start a new life cycle (Cabrales-Vargas, 1991). Twelve described species integrate the genus: *Trichobaris major* Barber 1935, *T. soror* Champion 1909, *T. pueblana* Casey 1920, *T. trinotata* Say 1831, *T. insolita* Casey 1892, *T. championi* Barber 1935, *T. mucorea* LeConte 1854, *T. bridwelli* Barber 1935, *T. compacta* Casey 1892, *T. pellicea* Boheman 1844, *T. texana* LeConte 1876 and *T. cylindrica* Casey 1892. Of these species, six develop into fruits of *Datura*

species, three species into the stems of potato, tobacco and tomatillo (*Solanum tuberosum, Nicotiana attenuata* and *Physalis* spp., respectively), and four into the stems of wild species of *Solanum* (*S. eleagnifolium, S. rostratum, S. carolinense*) (Barber, 1935). The life cycle of these weevils is closely associated with their host plants; for instance, the larvae of *T. bridwelli* cannot survive in a different host (Cuda and Burke, 1991). Ever since Barber's (1935) monographic study, the genus has not been studied again in such depth. His work on *Trichobaris* includes specimens from a wide range of locations and detailed morphological descriptions. Nevertheless, Barber (1935) noted issues not resolved as of yet, including the need for precise information on host plants and the relevance of body shape and size in delimiting morphological species because some named species were thought to be environmentally related variants. Thus, the study of speciation makes it necessary to identify and establish the species, their host plants and the determination of their evolutionary relationships.

In this study, we follow two approximations to study the evolution of *Trichobaris* species with their host plants. Under a phylogenetic framework, we tested whether the rostrum of *Trichobaris* species evolved according to the plant tissue used to oviposit, i.e., short rostrum for dig the stem in ancestral species of *Trichobaris* and long rostrum for dig the fruit in derived species of *Trichobaris*. We analyze the general morphology and, in particular, the rostrum of this weevils and build a molecular phylogeny using four gene sequences. We also tested whether diversification of *Trichobaris* is due mainly to the use of *Datura* species. To do this, under a phylogeographic framework, we map the host distribution on the haplotype network of each *Trichobaris* species and reconstructed the ancestral host plant species on the calibrated COI haplotype phylogeny to infer the historical associations or new colonizations of host plants.

2. Material and methods

2.1. Sampling and specimens examined

A total of 168 insects (including adult and larval stages) were collected in 33 localities across the *Trichobaris* distribution range in Mexico and the United States of America (USA) (Table 1; Fig. 1). All adult insects were identified using the key provided by Barber (1935). We collected almost all described species of *Trichobaris* Le Conte (Barber, 1935). Collectively, we found weevils in eight different species of *Datura*, four wild species of Solanaceae (*Physallis* sp., *Solanum eleagnifolium, S. carolinense* and *S. rostratum*) and one cultivated species of Solanaceae (potato, *S. tuberosum*).

We do not use all collected specimens of *Trichobaris* for the analysis because immature stages cannot be used to identify species. Larvae were used only if adults were not found in some localities. Due to their small size, non-destructive DNA extraction was not possible. Then, we randomly selected subsets of insects for morphology and genetic analysis. We used 139 insects for morphological analysis (Table 1), 156 insects for phylogenetic analysis (documenting geographical distribution and plant host), and 189 COI sequences generated in this study for phylogeographic analyses. In addition, we included the sequences obtained from two previous studies (*T. soror*, De-la-Mora et al., 2015; and *T. compacta*, De-la-Mora et al., in revision), resulting in a total of 844 COI sequences that comprise 198 COI haplotypes used to build a calibrated phylogeny and reconstruct the ancestral host plant.

2.2. Geometric morphometrics

A total of 139 insects were identified (Barber, 1935) and photographed from frontal (rostrum), lateral (body shape) and dorsal (pronotum) views. We oriented the images identically: the frontal (with the head at the middle of the photograph), the lateral always was the left side (with the mesopleuron placed in the middle) and the dorsal view, with the scutellum oriented towards the bottom and the head towards the top). We use two different imaging methods: a SteREO Discovery V8 Zeiss stereomicroscope combined with AxioVision software for image processing (Instituto de Ecología, UNAM) and a Canon EOS 6D camera mounted on StackShot automated macro rail ® Cognisys Inc., combined with Helicon Focus image processing software (Museum of Comparative Zoology, Harvard University).

Landmark analysis – We assessed three images per specimen for a total of 79 landmarks: 8 frontal, 39 lateral and 28 dorsal (Fig. 2). These were quantified from a set of two-dimensional coordinates with tpsDIG 1.4 software (Rohlf, 2004). To remove differences due to scale, orientation and location from landmark configurations, we used a minimum Procrustes distance criterion, implemented in MorphoJ (Klingenberg, 2011), to produce a set of partial Procrustes superimpositions of the specimens. The coordinates obtained were transformed into relative warp scores to generate a W matrix. By means of principal component analysis (PCA), we analyzed shape variation among morphotypes. To maximize individual differences according to species, we performed a canonical variate analysis followed by a discriminant analysis among pairs of species. Both analyses were implemented in MorphoJ (Klingenberg, 2011). Centroid size for frontal (rostrum), lateral (body shape) and dorsal (pronotum) views were plotted using R (R Development Core Team 2011).

2.3. DNA extraction, amplification and sequencing

Genomic DNA was extracted from whole insects using the DNeasy Blood & Tissue kit (Qiagen). Four gene sequences were used to build the *Trichobaris* phylogeny: two mitochondrial genes (CO1 and 16S rRNA) and two nuclear genes (18S and 28S rRNA). The primers used were COA3107 and COS2183N (Sota et al., 2004), LR-J-12887F and LR-N-13398R (Simon et al., 1994), 28S and 28SR, and Insect18S-midF and Insect18S-midR (from Kobayashi et al., 2012) for the COI, 16S, 28S and 18S genes, respectively. All PCR reactions were performed in volumes of 19.8 μL: 2 μL of 10x buffer, 1 μL of 20x $MgCl_2$, 0.8 μL of 10 mM dNTPs, 1 μL of 10 mM forward primer, 1 μL of 10 mM reverse primer, 1 μL of Taq Amplificasa®, 1 μL of 50 mM DNA and 12 μL of H_2O. The amplification conditions were the same for all gene sequences except for 16S rRNA, whose annealing temperature was 50 °C (Sota et al., 2004). An initial period of 5 min at 95 °C followed by 35 cycles of 1 min at 94 °C, 1.2 min at 55 °C and 1 min at 72 °C, with a final extension of 7 min at 72 °C. Samples were sequenced at the University of Washington's High-Throughput Genomics Center (ABI 3730XL sequencer and the ABI Prism kit Cycle Sequencing Big Dye Terminator Ready Reaction; Applied Biosystems).

Sequence processing was performed with Sequencher® 5.0 software (Gene Codes Corporation). The alignment was carried out by the FFT-NS-1 progressive alignment method, using the online version of the software MAFFT v7 (http://mafft.cbrc.jp/alignment/server/).

2.4. Estimation of genetic variability and phylogenetic analysis

The amount of genetic variability of COI, 16S, 18S and 28S sequences was estimated with the software DNAsp ver. 5.1 (Rozas et al., 2003). The total number of haplotypes, mutations, segregating sites (S), nucleotide diversity (π), haplotype diversity (h) and theta (θ) using Nei's (1987) equations were estimated for the mitochondrial (COI and 16S) and nuclear (18S and 28S) sequences.

Phylogenetic analyses were conducted for each locus separately, as well as for the concatenated matrix. *Sitophilus oryzae* (Curculionidae) was specified as the outgroup. Different codon positions were treated as separate partitions only for the COI sequence. Phylogenies were estimated using Bayesian Inference with the BEAST2 software. v2.4.5. (Bouckaert et al., 2014). The analysis was executed using 100,000,000 MCMC, 10,000 "burn in" with the GTR + I molecular replacement model using a fixed substitution rate of 1 substitution/site/unit time. The MCMC convergence of the chains was evaluated considering the

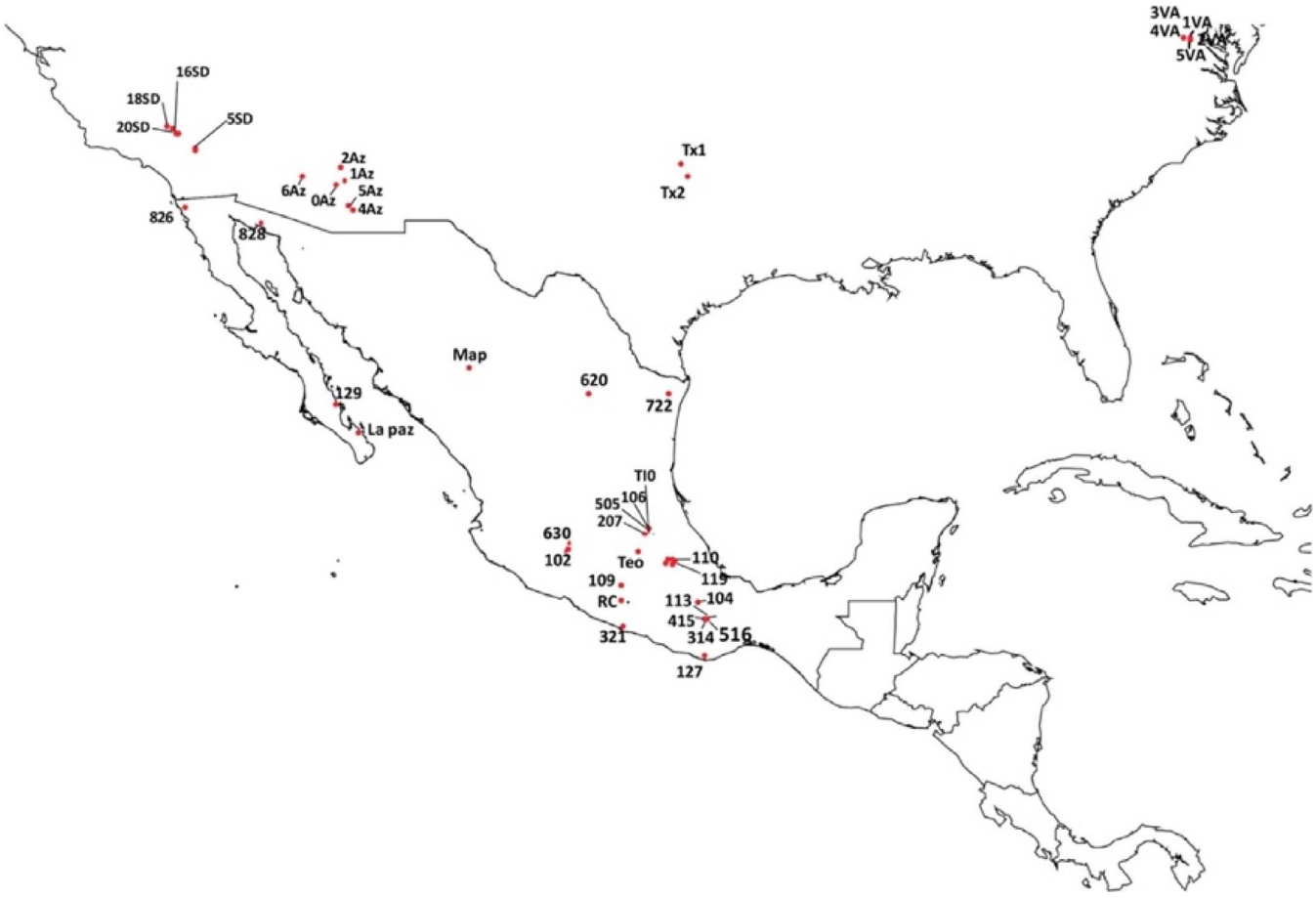

Fig. 1. *Trichobaris* sampling localities in USA and Mexico. Labels represent sample sites as indicated in Table 1.

effective sample size scores, all above 100, using Tracer v. 1.6 (Rambaut et al., 2014), and the trees were summarized with TreeAnnotator v2.1.2 (Rambaut and Drummond, 2014). Finally, the phylogenetic tree was visualized in FigTree v 1.4 (Rambaut, 2012).

2.5. Ancestral plant tissue and rostrum evolution

Second and third phylogenies were built using just one sequence per species (including the four markers), randomly chosen from the previous phylogenetic clustering (defined in geometric morphometric analysis). For the reconstruction of the ancestral plant tissue used by *Trichobaris*, all species were included, except for the phylogeny built for rostrum evolution, where only species with geometric morphometric information available for the rostrum of both males and females were used. Both phylogenies were estimated using Bayesian Inference with the BEAST2 software v2.4.5. (Bouckaert et al., 2014). The analysis was executed using 100 000 000 MCMC, 10 000 "burn in" with the GTR + I molecular replacement model using a fixed substitution rate of 1 substitution/site/unit time. Here, the MCMC convergence of the chains was also evaluated considering the effective sample size scores (ESS), all above 100, using Tracer v. 1.6 (Rambaut et al., 2014), and the trees were summarized with TreeAnnotator v2.1.2 (Rambaut and Drummond, 2014).

To reconstruct the ancestral plant tissue and the evolution of rostrum of *Trichobaris*, we reconstructed ancestral states with maximum likelihood using the "phytools" package (Revell, 2012), implemented in R (R Development Core Team 2011). The plant tissue was coded as a discrete characteristic (F = fruit, S = stem and U = for uncertainty of the tissue for the outgroup). We used the ER model (equal rates) that assumes the same probability for rate index matrix and the ARD model (all rates different) that assumes the different probability. A likelihood ratio test (LRT) was conducted to test for the best fit of the two models to the data. For the rostrum evolution, we use the mean centroid size per species, per sex, and coded it as a continuous characteristic. Here, we apply a Brownian motion model for the evolution of the rostrum. The phylogenetic signal of the rostrum per sex was estimated using the *K*-statistic (Blomberg et al., 2003) and λ (Pagel, 1999), both with nsim = 1 000 in "phytools" (Revell, 2012).

2.6. COI haplotype network and host plant

Using the gene variation at the COI gene sequence from two previous studies (De-la-Mora et al., 2015 and De-la-Mora et al., submitted) and the ones obtained in this study, we compared the measures of genetic diversity within and between species with the DNAsp software (Rozas et al., 2003). The relationship between haplotypes and their host-plant distribution was explored with haplotype networks built in Network ver. 4.6.1.1 software (Bandelt et al., 1999) through a median-joining algorithm.

2.7. COI haplotype phylogeny

Using the COI haplotypes of all *Trichobaris* species, we built the haplotype tree with Bayesian Inference using BEAST software. ver. 1.4.7 (Drummond and Rambaut, 2007). The analysis was executed using 10,000,000 MCMC, 1000 "burn in" with the GTR + I molecular replacement model. The phylogeny was calibrated with a 2% divergence per million years, previously reported for COI in coleopterans (Nakamine and Takeda, 2008). The MCMC convergence of the chains was evaluated considering the effective sample size scores (ESS), all

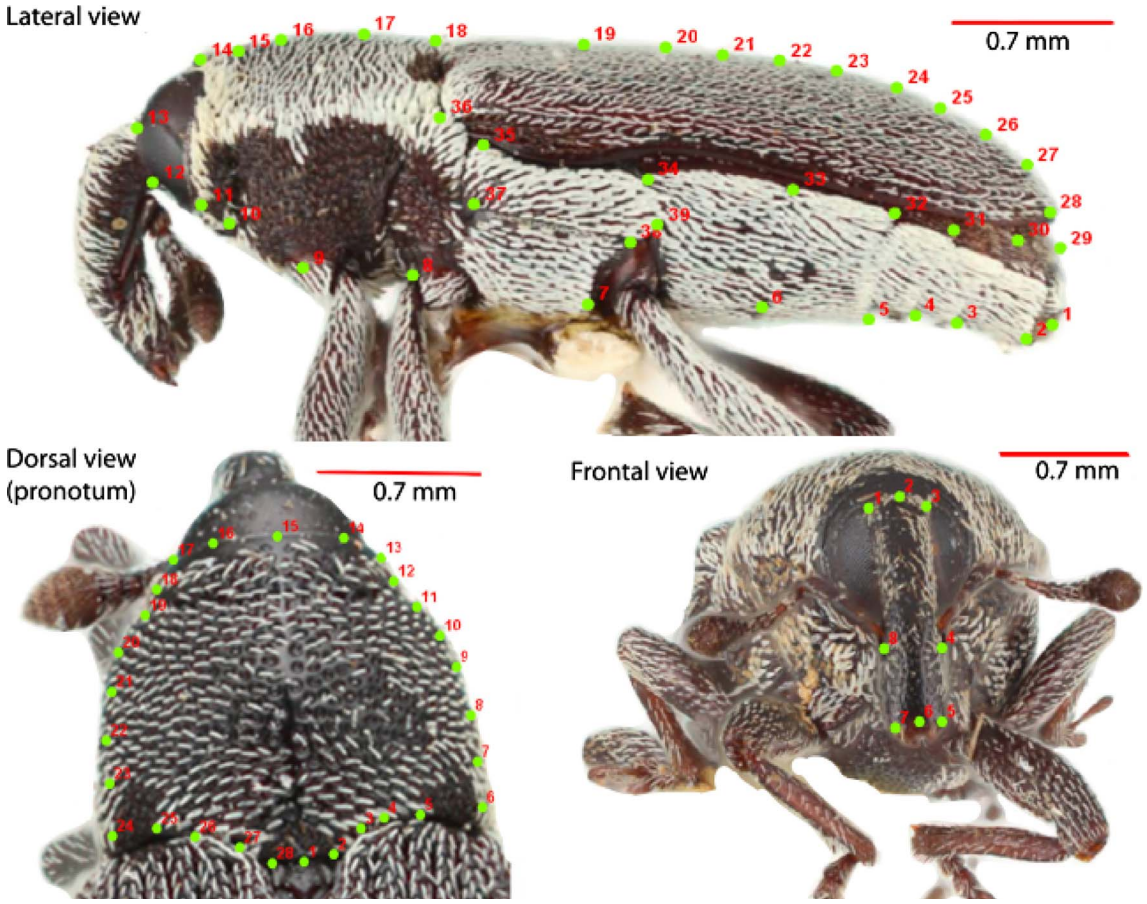

Fig. 2. Landmarks (green dots) used to perform the morphogeometric analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

above 100, using Tracer v. 1.6 (Rambaut et al., 2014), and the trees were summarized with TreeAnnotator v2.1.2 (Rambaut and Drummond, 2014). The phylogenetic tree was visualized in FigTree v 1.4 (Rambaut, 2012).

2.8. Ancestral host plant reconstruction analysis

The gene CO1 has been effective in recognizing insect haplotypes associated with host plants (Jurado-Rivera et al., 2009). Here, we used the phylogenetic tree built from COI haplotypes instead of the species phylogeny because the former has greater resolution, allowing for comparison of terminals to host plants. We coded ambiguous haplotypes (present in two or more host plants) present only in the most abundant host plant. Ancestral host utilization was estimated with maximum likelihood using the "phytools" package (Revell, 2012) implemented in R (R Development Core Team 2011). The eleven host plants occupied by *Trichobaris* species were considered a discrete character. The host plant of the outgroup species was required for the analysis, although this is an arbitrary assignment. We used a continuous-time Markov chain model (Mk model) (Suchard et al., 2001) for trait evolution, assuming all transitions in the character state with the same probability. We did not attempt to estimate the phylogenetic signal for discrete characters (some methods exist but for binary characters; see Fritz and Purvis, 2010). For ancestral host plant reconstruction, the geographic distribution of species using different hosts was also taken into account through the phylogeny of haplotypes. Different models of reconstruction were not tested because there is no empirical evidence or theoretical basis to suppose differential transition rates between states (host plants); for instance, some species of insects would follow secondary chemical compounds present in one species of plant and not in other. Additionally, it is methodologically complex to analyze 12 discrete states because of the large number of parameters involved.

3. Results

3.1. Species and geometric morphometric analysis

From sampled insects and according to Barber's descriptions, we identified *T. soror, T. major, T. compacta, T. mucorea, T. mucorea* var. *striatula* Casey 1920, *T. texana, T. cylindrica* and *T. trinotata.* A total of nine *Trichobaris* species were included in the geometric morphometric analysis, with eight species being distinguishable: *T. soror, T. major, T. mucorea, T. mucorea* var. *striatula, T. texana, T. cylindrica, T. trinotata* and *T. compacta* (Fig. 3). Missing from Barber's descriptions were *T. pueblana, T. compacta* var. *retrusa* and *T. bridwelli*. We found sexual dimorphism in the rostrum; *T. soror, T. major* and *T. trinotata* have females with longer and thinner rostrums among the other females of *Trichobaris* species (Fig. 4). Here, *T. insolita* and *T. cylindrica* are not shown due to sample size (< 2); Figs. 5 and 1S Supplementary Material).

3.2. Estimation of genetic variability and phylogenetic relationships

We sequenced 663 bp of COI, 454 bp of 16S, 429 bp of 18S and 771 bp of 28S from 156 randomly selected insects. There was substantial variation in resolution among the four gene regions. For COI, we found 59 haplotypes, with $h = 0.874$, $\pi = 0.052$, $\theta = 0.064$ and $S = 26$. For 16S, 26 haplotypes were found, with $h = 0.762$, $\pi = 0.025$, $\theta = 0.06$ and $S = 6$. For the nuclear sequences 18S and 28S, we found

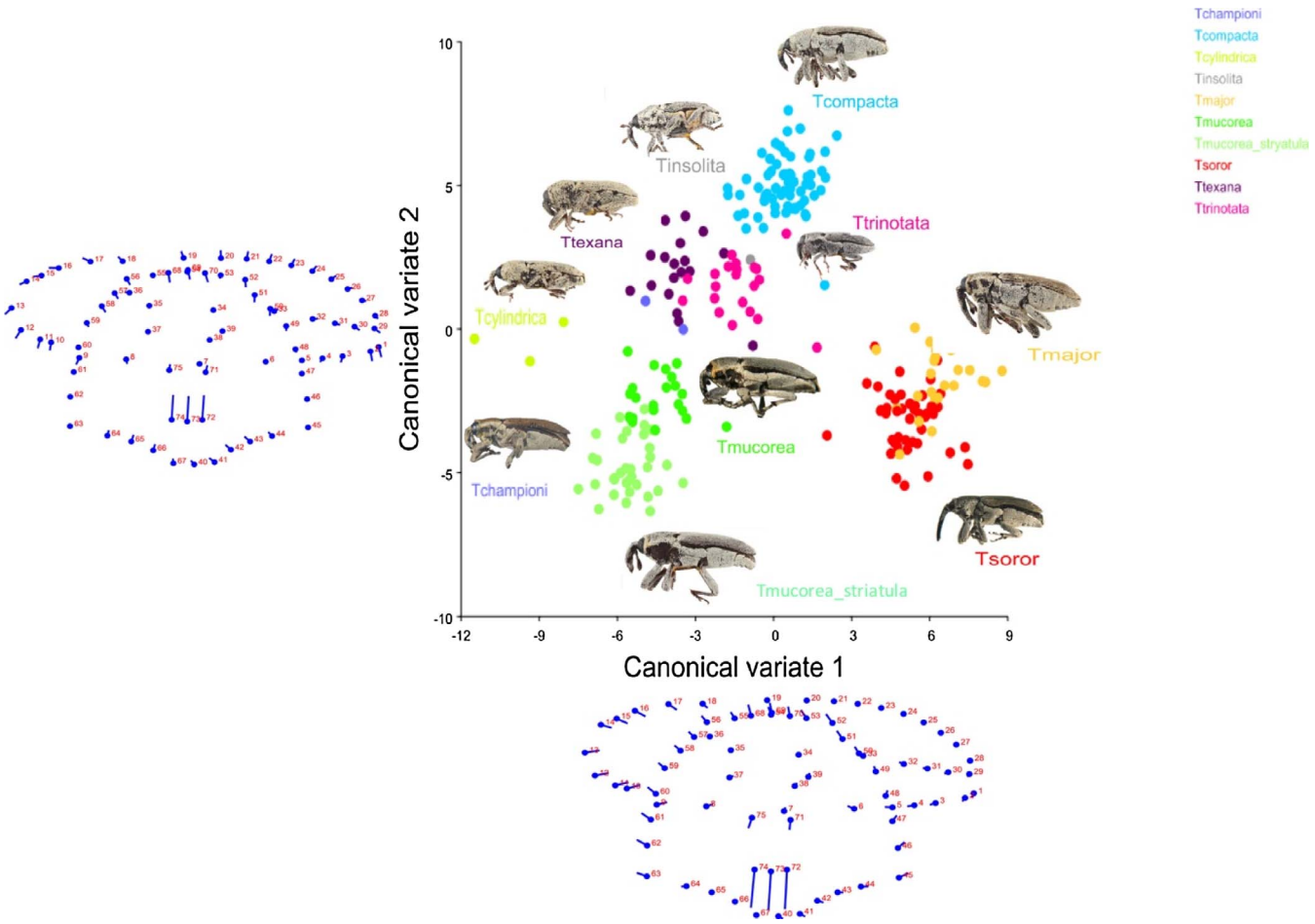

Fig. 3. Analysis of Canonical Variance of the genus *Trichobaris*, obtained by 75 landmarks from three photographs per specimen. Each individual is represented by a dot, coloured according to the species to which it belongs. The photograph is representative of each species. The axes are the canonical variate 1 and 2 that represent the changes in shape.

15 ($h = 0.593$, $\pi = 0.005$, $\theta = 0.020$ and $S = 3$) and 18 ($h = 0.518$, $\pi = 0.025$, $\theta = 0.062$ and $S = 10$) consensus sequences, respectively. GenBank accession numbers: KY984501-KY984658 for 28S, KY984659-KY984816 for 18S, KY984817-KY984974 for 16S and KX359724-KX359774 for COI.

The Bayesian phylogeny provides an estimate of the evolutionary relationships among *Trichobaris* species (Fig. 6; for posterior probability of parameters and tracer, see Fig. S2 Supplementary Material). Six species agree with the morphological groupings and species reported. *Trichobaris major* and *T. soror* belong to the same morphological group and are genetically indistinguishable. Because larvae were used for this analysis, identification of some species required further confirmation, namely, *T. pellicea*, which was identified on the basis of its distribution and host plant. In general, the phylogenetic tree shows high posterior probability support for eight *Trichobaris* species.

3.3. Host plant tissue and weevil's rostrum evolution

We reconstructed the host plant tissue used by weevils on the phylogeny of *Trichobaris* (Fig. 6; for posterior probability of parameters and tracer see Fig. S3 Supplementary Material). Two clades use different plant tissues to oviposit: Clade 1 (*T. cylindrica* and *T. texana)* uses the stem, while clade 2 (*T. pellicea, T. mucorea* var. *striatula, T. compacta, T. mucorea, T. soror* and *T. trinotata*) uses the fruit, except *T. trinotata*. Models ER and ARD for the rate of transitions (LRT = 4.20: critical value = 11.7 for $p = 0.05$) were not significantly different. Rostrum varies from 1.32 to 2.24 mm for females and from 1.33 to 1.66 for males (Fig. 7; for posterior probability of parameters and tracer see Fig. S4 Supplementary Material). The phylogenetic signal for this trait was low in both sexes (females: *K*-statistic = 0.1382412, *p*-value = 0.373; λ = 6.843901e-05, *p*-value = 1; males: *K*-statistic = 0.2773729, *p*-value = 0.477; λ = 6.843901e-05, *p*-value = 1).

3.4. Phylogeographic analysis

We obtained eight haplotype networks of 189 haplotypes (Fig. 8) using the variation of only the COI gene sequence (Table 2) of 844 insects, including the samples used for the estimation of the phylogeny (see above).

The haplotype network of *T. mucorea* (n = 67) shows 16 haplotypes (Fig. 6S Supplementary Material), most of them distributed only on *D. stramonium,* three on *D. inoxia* and one on three host plants: *D. pruinosa, D. discolor* and *D. stramonium* (Fig. S6 Supplementary Material). The haplotype network of *T. soror* (n = 469) shows 99 haplotypes, mostly on *D. stramonium,* but nine haplotypes are also found on *D. quercifolia.* The low-frequency haplotypes So92, So93 and So94 were found on *D. inoxia, D. pruinosa* and *Solanum carolinense* (Fig. S7 Supplementary Material). The haplotype network of *T. trinotata* (n = 21) shows four haplotypes with a shared distribution on *S. carolinense, S. tuberosum* and *D. stramonium* (Fig. S8 Supplementary Material). The haplotype network of *T. compacta* (n = 232) shows 49 haplotypes, most of which were sampled on *D. wrightii.* Five haplotypes are distributed on *D. reburra*, eight on *D. discolor*, two on *D. pruinosa* and two in *D. inoxia* (Fig. S9 Supplementary Material).

The haplotype network of *T. mucorea* var. *striatula* (n = 23) shows 13 haplotypes mainly distributed on *D. wrightii* and four haplotypes on

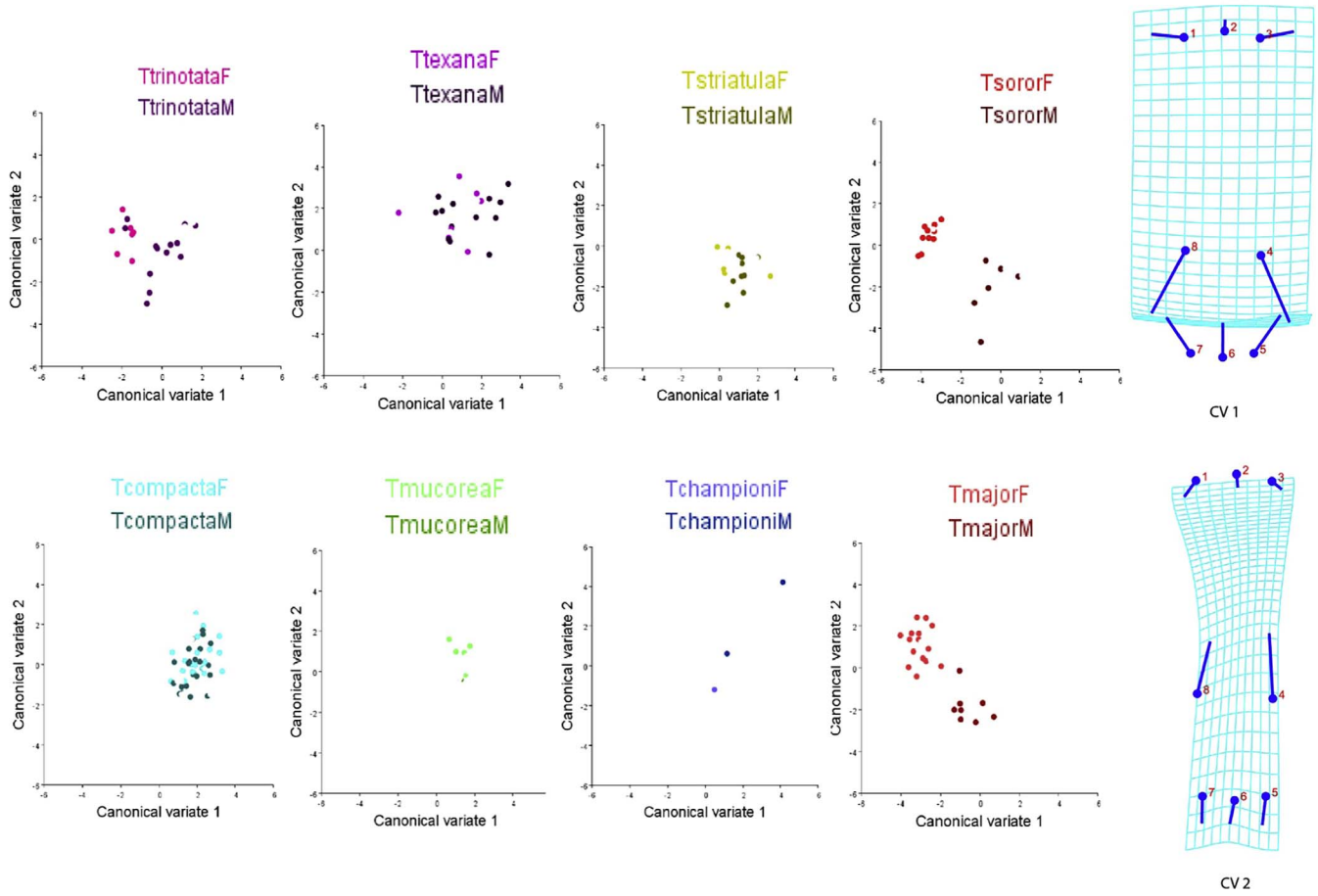

Fig. 4. Analysis of Canonical Variance of the rostrum of species of genus *Trichobaris*, obtained by eight landmarks per specimen. Males (M) and females (F) of each species are represented by the same colour darkest and lightest, respectively. The axes are the canonical variate 1 and 2 that represent the changes in shape represented on the transformation gif outside of the graphic.

D. discolor (Fig. S10 Supplementary Material). The haplotype network of *T. texana* (n = 7) shows three haplotypes distributed on *S. rostratum* (Fig. S11 Supplementary Material). The haplotype network of *T. cylindrica* (n = 4) shows three haplotypes distributed on *S. eleagnifolium* (Fig. S11 Supplementary Material). Finally, *T. pellicea* n = 3 shows two haplotypes distributed on *D. stramonium* (Fig. S11 Supplementary Material).

3.5. Haplotype calibrated phylogeny

Most clades that were inferred with high support based on the four loci (Fig. 6) were also inferred and strongly supported by the COI haplotype phylogeny (Fig. 9; for posterior probability of parameters and tracer see Fig. S5 Supplementary Material), except for *T. pellicea*, which appears in a different position. The time-calibrated haplotype phylogeny shows that most *Trichobaris* species are no older than 6.6 (± 1.5) million years ago (Ma). Diversification of *T. soror, T. trinotata, T. mucorea, T. compacta* and *T. mucorea* var. *striatula* haplotypes is very recent (less than 0.5 Ma) (Fig. 9).

3.6. Estimation of ancestral host plant species

We estimated the ancestral host plant based on the COI haplotype phylogeny of *Trichobaris* species (Fig. 9). Here, we indicated the ancestral host at eleven nodes from (0–10), whose estimated likelihood values were not conclusive for nodes 0, 1, 2 and 4. Node 3 suggests with a 0.528 likelihood that *S. eleagnifolium* could be the ancestral host plant of *T. texana* and *T. cylindrica*. Likelihood values of 0.318 and 0.982 from nodes 5 and 7, respectively, support the hypothesis that *D. wrightii* was the ancestral host plant of *T. compacta* and *T. mucorea* var. *striatula*. Finally, nodes 6, 8, 9 and 10 suggest that *D. stramonium* was the ancestral host plant of *T. soror, T. trinotata* and *T. mucorea* (0.272, 0.784, 0.862 and 0.983, respectively, at each node).

4. Discussion

This study constitutes the first integrative analysis, using morphological, phylogenetic and phylogeographic approaches, of *Trichobaris* species to investigate their association with Solanaceae plants. Our results provide relevant insights to understand the evolution of *Trichobaris* and its relationships with host plants.

4.1. Trichobaris species and Barber's key

Barber's monographic account of *Trichobaris* (Barber, 1935) recognizes female rostrum length, body shape and male genitalia as critical morphological characters to distinguish *Trichobaris* species. In Barber's key, the female rostrum allows us to distinguish *T. soror, T. pueblana* and *T. major* from all other *Trichobaris* species. In our study, we found that male and female rostrum shape is an important character across *Trichobaris* species. The rostrum has been identified as a key innovation in the evolution of weevils (Oberprieler et al., 2007), and even at a lower taxonomic level, the rostrum is an important trait in the evolution of this genus (Fig. 7).

Evidence indicates that *T. soror, T. major, T. trinotata, T. compacta, T. texana* and *T. cylindrica* differ in body shape from each other (Fig. 3). In

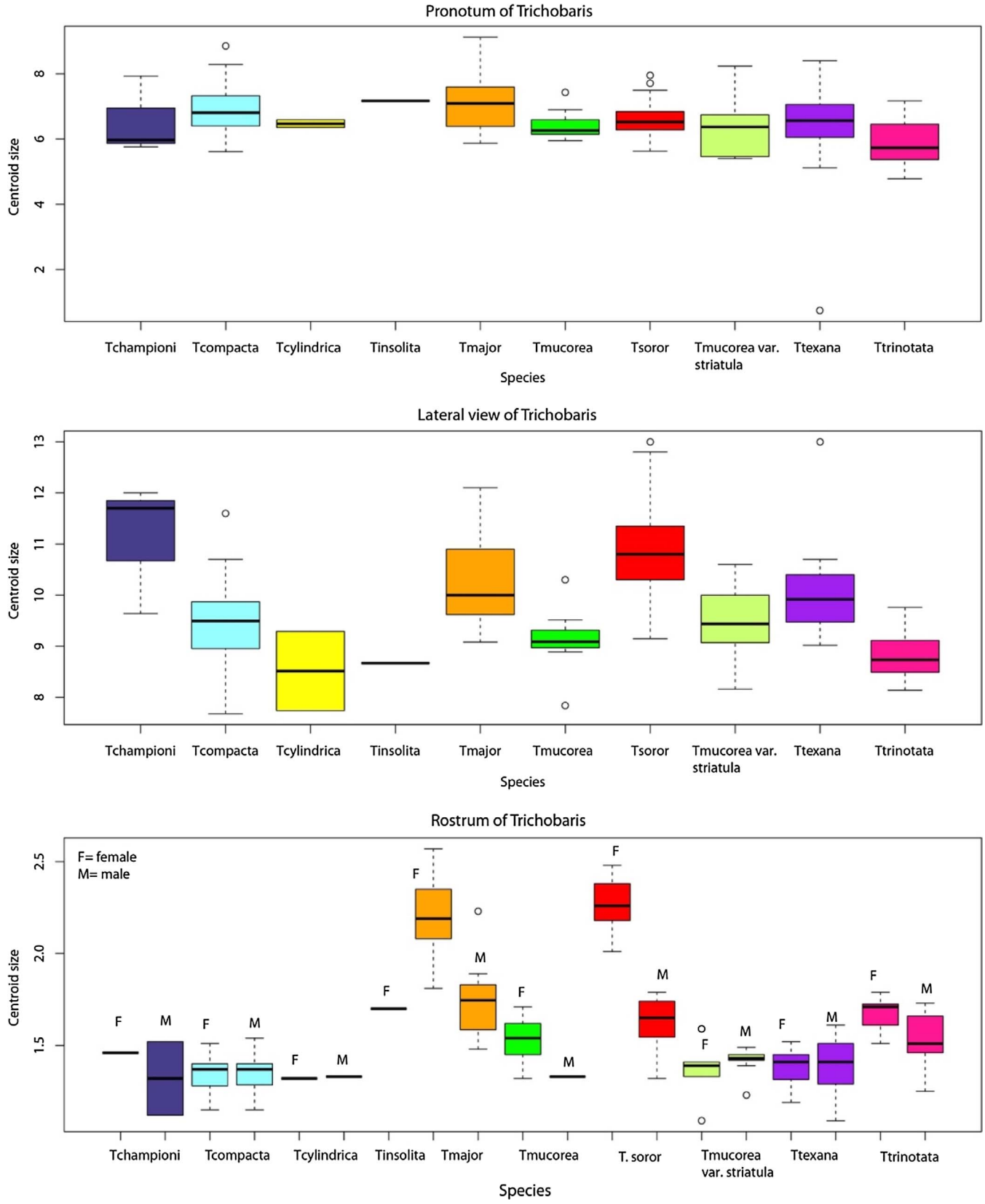

Fig. 5. Centroid size of *Trichobaris* species. Panels: Upper) Dorsal view or *pronotum*. Middle) Lateral view or body shape. Lower) Rostrum by sex of *Trichobaris* species. F = females, M = Males.

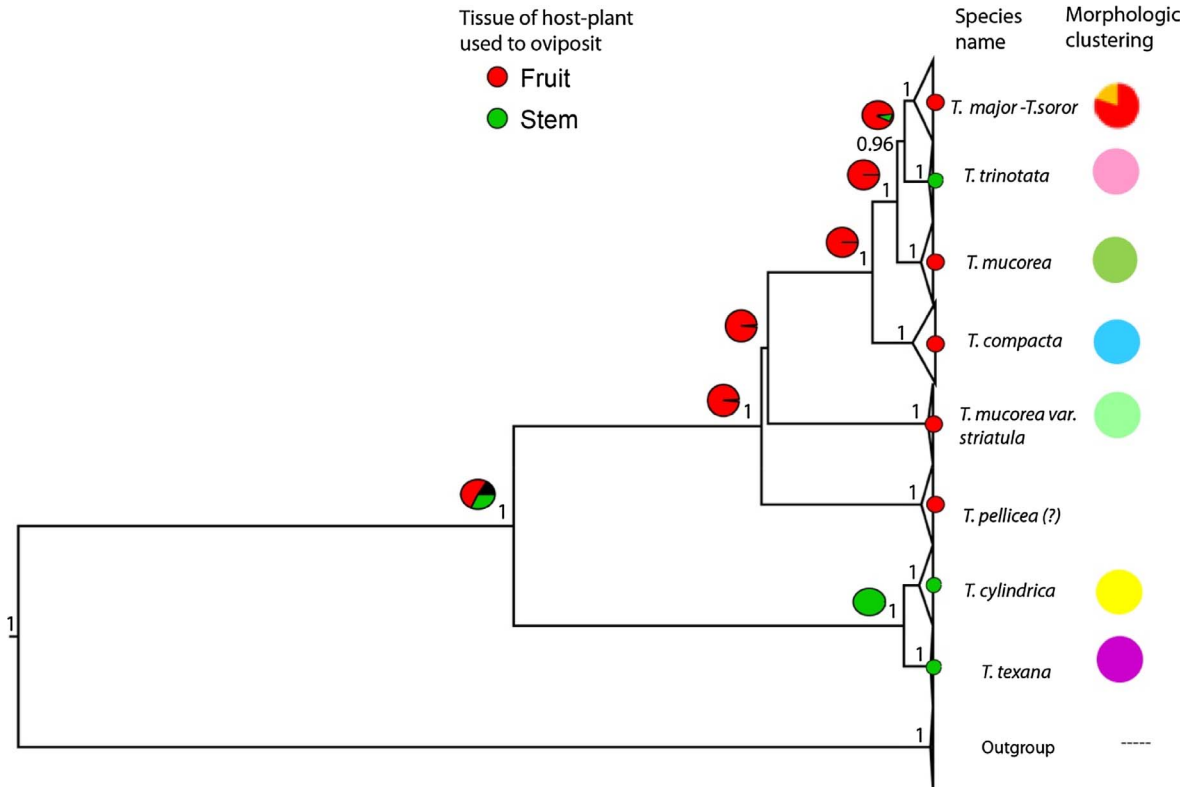

Fig. 6. Bayesian phylogeny of *Trichobaris*. Phylogeny of *Trichobaris*, estimated by Bayesian Inference using 18S and 28S nrDNA and the mitochondrial 16S and COI. The morphological clustering from canonical variance analysis is indicated with the circles after species names. (?) indicates that the species identity could not be corroborated by morphology. Red and green circles on tips are observed values of plant tissue used by weevils species to oviposit and circles at nodes are the ancestral states reconstructed by maximum likelihood. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

species such as *T. insolita* and *T. championi*, the clustering of individuals had no statistical significance, although this might be a consequence of small sample sizes.

We found that *T. soror* and *T. major* are morphologically distinguishable (Fig. 6), although they do not show differences in COI haplotypes (Fig. 3). In contrast, *T. mucorea* and *T. mucorea* var. *striatula* show close morphological clustering (Fig. 3), but they are genetically very distant, similar to cryptic species; in fact, *T. mucorea* is closely related to *T. soror*, whereas *T. mucorea* var. *striatula* is closer to *T. compacta* (Fig. 3).

Barber's species of *Trichobaris* not included in our phylogeny were *T. pueblana, T. championi, T. bridwelli* and *T. insolita.* Although we sampled several populations in the state of Puebla and in the reported host plant *(D. stramonium),* we did not find *T. pueblana*. *Trichobaris championi* is a crop pest of tomatillo (*Physalis ixocarpa*) in Mexico (Calyecac-Cortero et al., 2004). Instead, the weevils collected in wild *Physalis* species

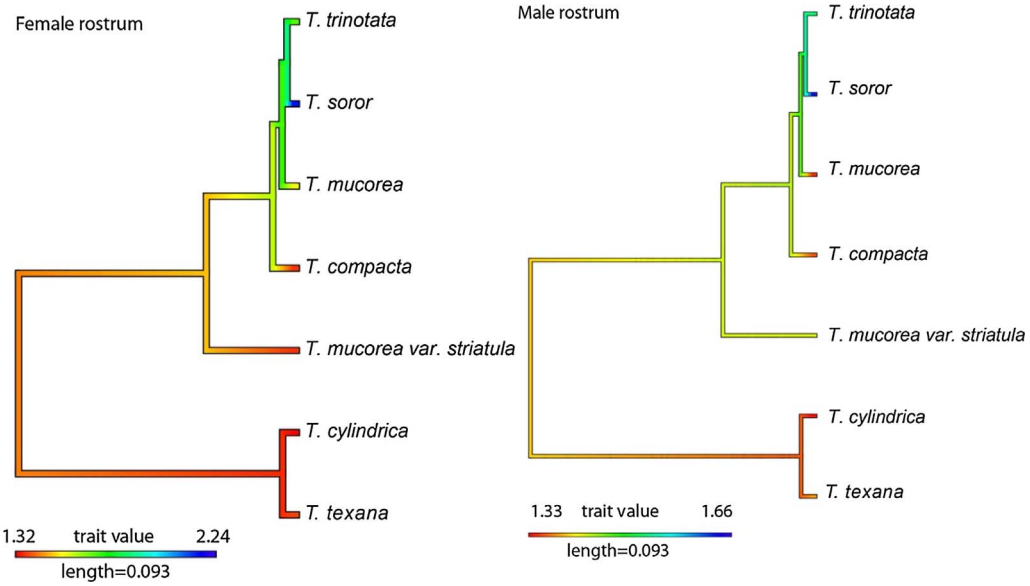

Fig. 7. Colour gradient projection of rostrum, observed and reconstructed size, onto the phylogeny of *Trichobaris* species. Estimated by maximum likelihood under a Brownian motion model of evolution.

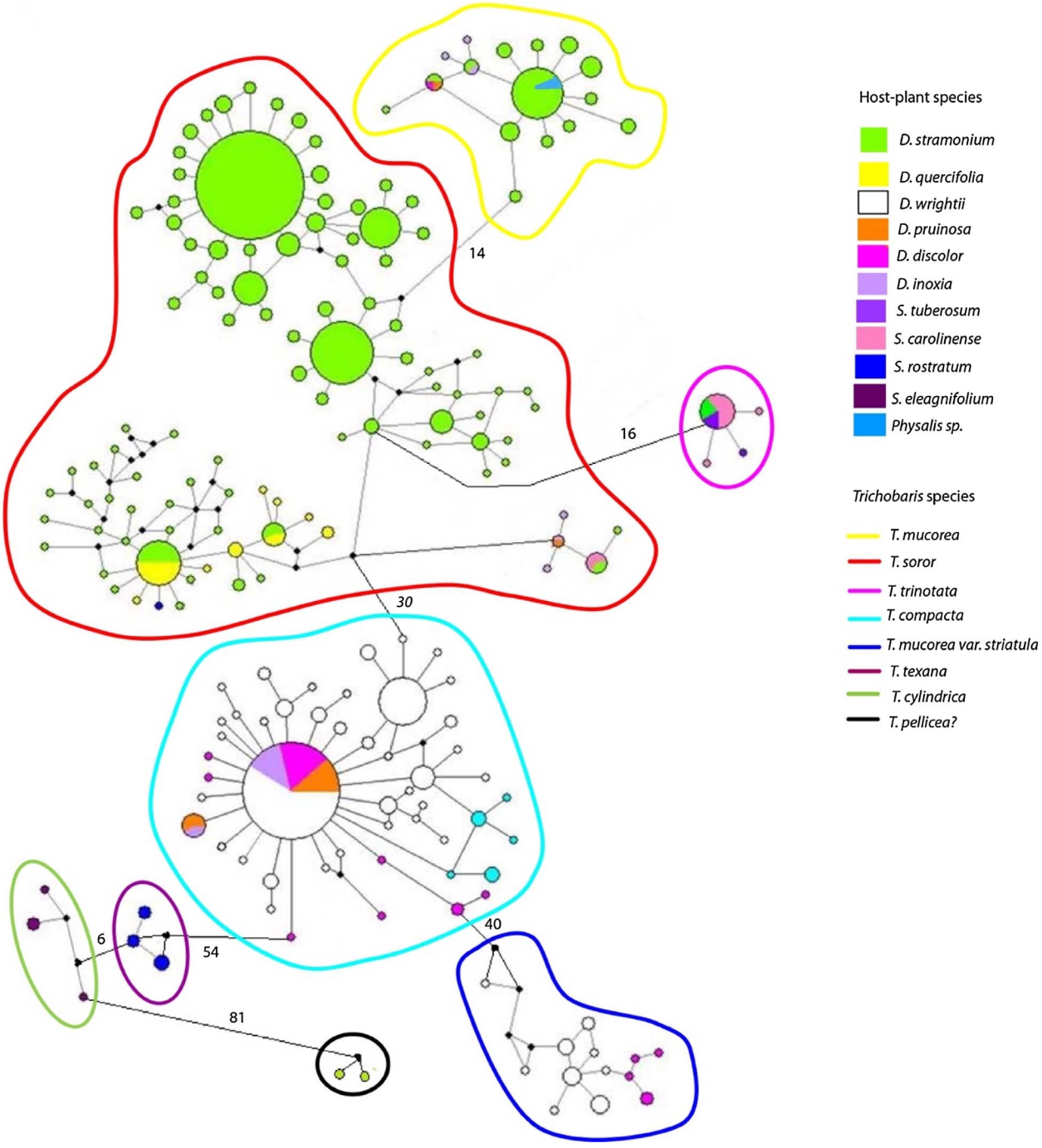

Fig. 8. Haplotype network of *Trichobaris* species estimated using the COI gene (663 bp) using the Median joining algorithm. Black dots represent vectors and the numbers on branches indicate mutational steps (see Supplementary Material for a more detailed view, in Figs. S6–S11). *Trichobaris pellicea*? Indicates that this species was not corroborated by morphology.

Table 2
Genetic diversity for *Trichobaris* species using 663 bp of COI gene.

Species	# insects	# haplotypes	h
T. soror	469	99	0.785
T. trinotata	21	4	0.271
T. mucorea	67	16	0.701
T. compacta	232	49	0.709
T. mucorea var. striatula	23	13	0.932
T. texana	7	3	0.761
T. cylindrica	4	3	0.833
T. pellicea (?)	3	2	0.667
Total	826	189	0.905

belonged to *T. mucorea,* which has also been reported in the stems of *Nicotiana attenuata* (Diezel et al., 2011). *Trichobaris bridwelli* was reported by Barber as the single weevil species that feeds upon the fruits of *D. stramonium* in the USA (Barber, 1935); however, the species of *Trichobaris* that we collected on *D. stramonium* was *T. soror* (see below). Finally, a single *T. insolita* was reported from a single locality in Florida (USA) on a patch of plants of *Physalis sp*. (Barber, 1935). We were not able to corroborate that finding.

4.2. Phylogenetic patterns and rostrum evolution

In *Curculio* species (Toju and Sota, 2009; Davis, 2014), an evolutionary trend to increase rostrum length in specialized relationships

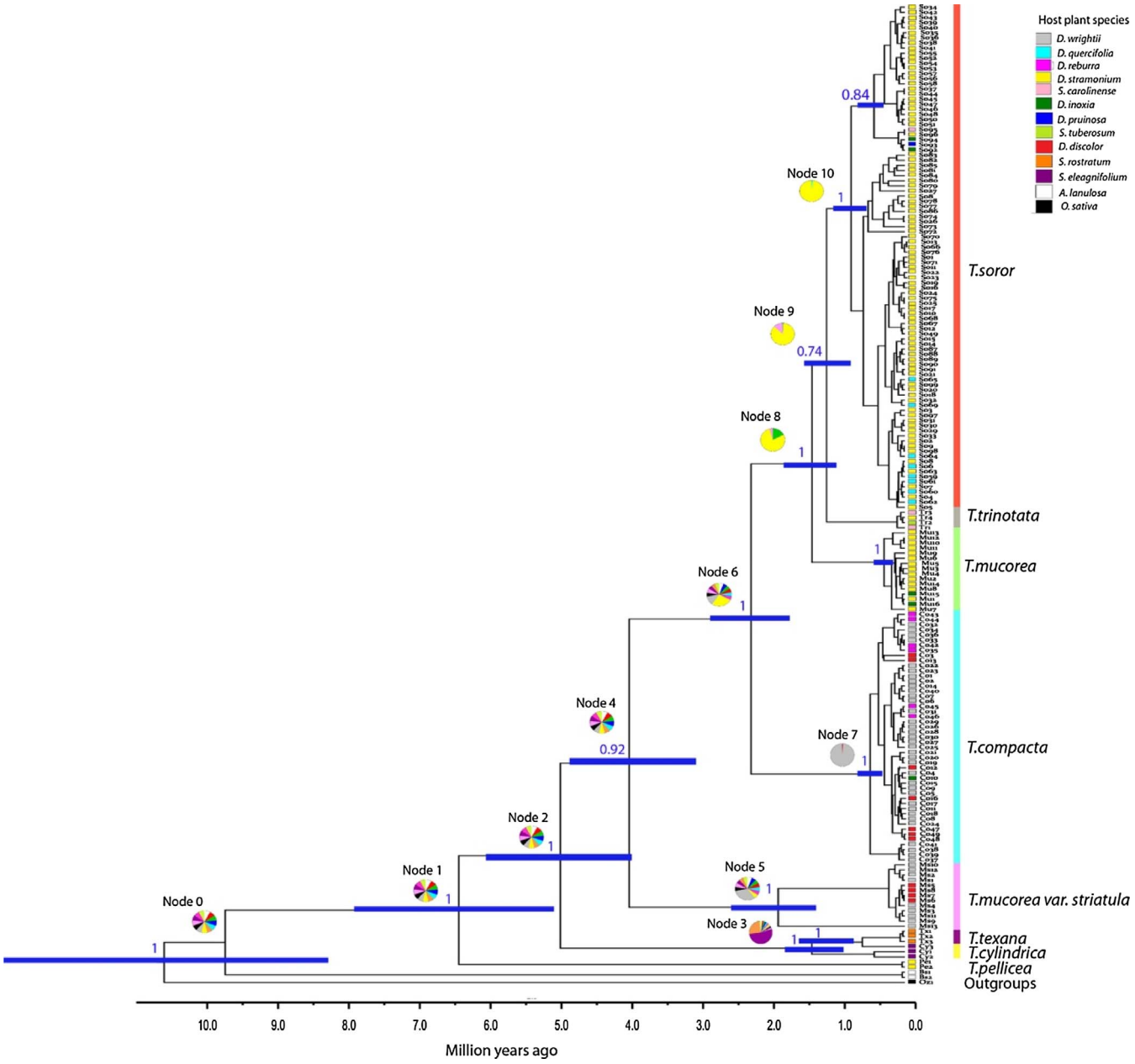

Fig. 9. Calibrated COI haplotype tree of *Trichobaris* species. Node bars represent the variation on age estimation. Blue numbers on branches indicate node support (posterior probability). The estimation of the ancestral host plant was carried out in each node of *Trichobaris* haplotype tree using maximum likelihood; the pie charts illustrate the relative likelihood of each of 13 possible host plants. The coloured box at the tips are the plants where the haplotype was collected; each *Trichobaris* species is indicated by lateral bars at right side. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with their host plants to drill the fruits and oviposit has been documented (e.g., Toju, 2009). In *Trichobaris*, we observed a tendency to increase rostrum size among weevil species that use fruits, instead of stems, to oviposit (Fig. 6) in comparison with the ancestral clade (*T. texana* and *T. cylindrica*); however, this remains speculative at the moment. When the phylogenetic signal is high, closely related species exhibit similar traits, and this biological similarity decreases as the evolutionary distance between species increases. In our study, rostrum size had a low phylogenetic signal, perhaps due to the bias induced by small branches near the tips (e.g., *T. trinotata* and *T. soror*).

4.3. Phylogeographic patterns and host plant associations

Although our sampling of *Trichobaris* did not cover all the geographic distribution regions reported for the species in the USA's midwest (*T. texana, T. bridwelli* and *T. trinotata;* Barber, 1935; Cuda and Burke, 1984), we report the host plant of these species. We found that the insects collected on *D. stramonium* in the USA (locality 1VA, Fig. 1 and Table 1) vary only in 2 or 3 mutational steps in the haplotype network of *T. soror* (haplotypes: So93, So94, So95 and So96; Fig. S7 Supplementary Material). Additionally, *T. bridwelli* is reported as the only weevil species associated with *D. stramonium* in the USA (Barber, 1935). Thus, this suggests that *T. soror* is also distributed up to the western USA. The reported potato pest, *T. trinotata,* is associated with *S. tuberosum* and *S. carolinense* (Cuda and Burke, 1986; Wise, 2007), and our study supports these observations (Fig. S8 Supplementary Material).

Mapping the host plant onto the haplotype network and the ancestral host estimation using the phylogeny reveal several relevant aspects (Figs. 8 and 9). First, the use of *Solanum* plants by *Trichobaris*

weevils could be older than the occupation of *Datura*, except for *S. carolinense* and *S. tuberosum*, whose estimated ancestral host is *D. stramonium*. Second, the use of different species of *Datura* by *T. compacta* may be more recent than its interaction with *D. wrightii*. Almost all beetles collected in *D. stramonium* were *T. soror*, which also uses *D. quercifolia*. *T. mucorea* is associated with *D. stramonium*, and *T. mucorea* var. *striatula* is associated with *D. wrightii* but has a clade within *D. discolor*. Inconclusive cases, possibly due to sample size, are *T. trinotata, T. texana, T. cylindrica* and *T. pellicea*. This means that historical associations and recent colonizations are both critical in explaining the relationship of *Trichobaris* with its host plants.

A possible directionality on the evolution of resource use in weevils has been proposed (Malvardi et al., 2002). For *Trichobaris* species, we found that the use of stems and fruits is bidirectional. In the case of *T. trinotata*, we found that this species develops in the stems of both *S. carolinense* and *S. tuberosum*. It is likely that its ancestor developed in *Datura* fruits (Fig. 6). It has been suggested that host shifts in closely related insect species are more correlated with host plant defensive secondary chemistry than with plant phylogeny (Futuyma and Agrawal, 2009: Becerra, 2015). In the case of *T. soror*, it is known that there is spatial variation in the levels of infestation of *D. stramonium* (Borbolla, 2015), likely associated with alkaloid production, namely, atropine and scopolamine (Miranda-Pérez et al., 2016). Future studies are needed to investigate the role of secondary compound chemistry in the association of *Trichobaris* with its plant hosts.

5. Conclusions

Here, we present the phylogenetic relationships among *Trichobaris* species based on four gene sequences. Additionally, we used a geometric morphometric approach for *Trichobaris* species to assess their concordance with phylogenetic clades and to test the hypothesis that *T. texana* and *T. cylindrica* have weak sexual dimorphism in rostrum size and use the stem of Solanaceae, probably since the origin of the genus (6 ± 1.5 Ma), whereas other, more recent, species of *Trichobaris* display sexual dimorphism in rostrum size (*T. soror* and *T. trinotata*, mainly) and use the fruits of *Datura* species (with only one possible reversion: *T. trinotata*, to the stem of *Solanum*).

Trichobaris soror and *T. major* belong to different morphological groups but to the same clade in the phylogeny, suggesting a recent divergence (less than 1 Ma). In contrast, *T. mucorea* and *T. mucorea* var. *striatula* are morphologically close but belong to different clades in the phylogeny, suggesting that they constitute different species. The latter warrants a formal description.

The haplotype network and the calibrated phylogeny built with the COI gene sequence suggest that most species of *Trichobaris* have originated recently in interaction with their host plants. Thus, our results indicate that *Trichobaris* species diverged very recently (less than 5 Ma), mainly associated with *Solanum* and *Datura* species, and that such interactions perhaps spurred the evolution of the rostrum to oviposit in different tissues.

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Conflict of interest

None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.02.018.

References

Aoki, K., Kato, M., Murakami, N., 2009. Phylogeographical patterns of a generalist acorn weevil: insight into the biogeographical history of broadleaved deciduous and evergreen forests. BMC Evol. Biol. 9, 103.
Aoki, K., Kato, M., Murakami, N., 2011. Phylogeography of phytophagous weevils and plant species in broadleaved evergreen forests: a congruent genetic gap between western and eastern parts of Japan. Insects 2 (2), 128–150.
Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA, pp. 447.
Avise, J.C., Johns, G.C., 1999. Proposal for a standardized temporal scheme of biological classification for extant species. Proc. Natl. Acad. Sci. 96 (13), 7358–7363.
Avtzis, D.N., Perlerou, C., Diamandis, S., 2013. Geographic distribution of chestnut feeding insects in Greece. J. Pest. Sci. 86 (2), 185–191.
Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16 (1), 37–48.
Barber, H.S., 1935. The tobacco and solanum weevils of the genus Trichobaris. Miscellaneous Publication No. 226. United States Department of Agriculture, Washington DC, 28 p.
Barr, N., Ruiz-Arce, R., Obregón, O., De Leon, R., Foster, N., Reuter, C., Vacek, D., 2013. Molecular diagnosis of populational variants of *Anthonomus grandis* (Coleoptera: Curculionidae) in North America. J. Econ. Entomol. 106 (1), 437–449.
Barraclough, T.G., Nee, S., 2001. Phylogenetics and speciation. Trends Ecol. Evol. 16 (7), 391–399.
Basio, R.G., Johnson, P.J., Pua, D.R., Bergonia, H.T., Diloy, C.C., Villegas, E.L., 1994. Mango pulp weevil [Sternochetus frigidus (Fabr.)](Curculionidae, Coleoptera) found in Palawan [Philippines]. Philippine. Entomologist 9.
Becerra, J.X., 2015. Macroevolutionary and geographical intensification of chemical defense in plants driven by insect herbivore selection pressure. Curr. Opin. Insect. Sci. 8, 15–21.
Bierig, A., 1939. El picudo del aguacate. The avocado weevil. Revista del Centro Nacional de Agricultura (Costa Rica) 4 (6), 355–357.
Blomberg, S.P., Garland Jr, T., Ives, A.R., 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57 (4), 717–745.
Borbolla, M., 2015. Estructura genética de Trichobaris soror depredador de semillas de Datura stramonium. B.Sc. Thesis, Biology, Faculty of Sciences, UNAM, Mexico City.
Briese, D.T., Espiau, C., Pouchot-Lermans, A., 1996. Micro-evolution in the weevil genus *Larinus*: the formation of host biotypes and speciation. Mol. Ecol. 5 (4), 531–545.
Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Drummond, A.J., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10 (4), e1003537.
Cabrales-Vargas, R.A., 1991. Demografía e historia natural de Datura stramonium L. en el Pedregal de San Angel con algunas implicaciones evolutivas. B.Sc. Thesis, Biology, Faculty of Sciences, UNAM, Mexico City.
Calyecac-Cortero, H.G., Cibrián-Tovar, J., Bautista-Martinez, N., López-Collado, J., 2004. Comportamiento de alimentación, cortejo, cópula y oviposición de *Trichobaris championi* Barber (Coleoptera: Curculionidae). Agrociencia 38, 365–373.
Capinera, J.L., 2005. Pepper weevil, Anthonomus eugenii Cano (Insecta: Coleoptera: Curculionidae). University of Florida. IFAS Extension. EENY-278.
Castañeda-Vildózola, Á., Franco-Mora, O., Alemán, J.C.R., Ruiz-Montiel, C., Váldez-Carrasco, J., Equihua-Martínez, A., 2015. New distribution records of the small avocado seed weevil, *Conotrachelus* perseae Barber (Coleoptera: Curculionidae), in Mexico and notes on its biology. Coleopterists Bull. 69 (2), 267–271.
Charlet, L.D., 1983. Distribution and abundance of a stem weevil, *Cylindrocopturus adspersus* (Coleoptera: Curculionidae), in cultivated sunflower in the northern plains. Environ. Entomol. 12 (5), 1526–1528.
Chen, W., Zhong, Z., Dai, W., Fan, Q., He, S., 2017. Phylogeographic structure, cryptic speciation and demographic history of the sharpbelly *(Hemiculter leucisculus*), a freshwater habitat generalist from southern China. BMC Evol. Biol. 17 (1), 216.
Cuda, J.P., Burke, H.R., 1984. Biology and impact of *Trichobaris texana* (Coleoptera: Curculionidae) on silverleaf nightshade, *Solanum elaeagnifolium* in Central Texas. Proc. VI Int. Symp. Biol. Contr. Weeds 19, 25.
Cuda, J.P., Burke, H.R., 1986. Reproduction and development of the potato stalk borer, (Coleoptera: Curculionidae) with notes on field biology. J. Econ. Entomol. 79 (6), 1548–1554.
Cuda, J.P., Burke, H.R., 1991. Biology of *Trichobaris bridwelli* (Coleoptera: Curculionidae), a possible agent for the biological control of *Datura stramonium* (Solanaceae). Environ. Entomol. 20 (3), 899–908.
Davis, S.R., 2014. Morphology, phylogeny, and evolutionary development in the weevils (Insecta: Coleoptera: Curculionoidea) (Doctoral Dissertation, University of Kansas).
De-la-Mora, M., Piñero, D., Núñez-Farfán, J., 2015. Phylogeography of specialist weevil *Trichobaris soror:* a seed predator of *Datura stramonium*. Genetica 143 (6), 681–691.
Diezel, C., Kessler, D., Baldwin, I.T., 2011. Pithy protection: *Nicotiana attenuata*'s jasmonic

acid-mediated defenses are required to resist stem-boring weevil larvae. Plant Physiol. 155 (4), 1936–1946.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7 (1), 214.
Ehrlich, P.R., Raven, P.H., 1964. Butterflies and plants: a study in coevolution. Evolution 586–608.
Farrell, B.D., 1998. "Inordinate fondness" explained: Why are there so many beetles? Science 281 (5376), 555–559.
Fritz, S.A., Purvis, A., 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. Conserv. Biol. 24 (4), 1042–1051.
Futuyma, D.J., Agrawal, A.A., 2009. Macroevolution and the biological diversity of plants and herbivores. Proc. Natl. Acad. Sci. 106 (43), 18054–18061.
Gavrilets, S., 2003. Perspective: models of speciation: what have we learned in 40 years? Evolution 57 (10), 2197–2215.
Grimaldi, D., Engel, M.S., 2005. Evolution of the Insects. Cambridge University Press, New York, pp. 755.
Hernández-Vera, G., Mitrovic, M., Jovic, J., Tosevski, I., Caldara, R., Gassmann, A., Emerson, B.C., 2010. Host-associated genetic differentiation in a seed parasitic weevil *Rhinusa antirrhini* (Coleptera: Curculionidae) revealed by mitochondrial and nuclear sequence data. Mol. Ecol. 19, 2286–2300.
Holt, B.G., Jønsson, K.A., 2014. Reconciling hierarchical taxonomy with molecular phylogenies. Syst. Biol. 63 (6), 1010–1017.
Iwase, S.I., Nakahira, K., Tuda, M., Kagoshima, K., Takagi, M., 2015. Host-plant dependent population genetics of the invading weevil *Hypera postica*. Bull. Entomol. Res. 105 (01), 92–100.
Jurado-Rivera, J.A., Vogler, A.P., Reid, C.A., Petitpierre, E., Gómez-Zurita, J., 2009. DNA barcoding insect–host plant associations. Proc. Royal Soc. London B: Biol. Sci. 276 (1657), 639–648.
Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Mol. Ecol. Resour. 11 (2), 353–357.
Kobayashi, C., Okuyama, Y., Kawazoe, K., Kato, M., 2012. The evolutionary history of maternal plant-manipulation and larval feeding behaviours in attelabid weevils (Coleoptera; Curculionoidea). Mol. Phylogenet. Evol. 64 (2), 318–330.
Kohyama, T.I., Matsumoto, K., Katakura, H., 2014. Deep phylogeographical structure and parallel host range evolution in the leaf beetle *Agelasa nigriceps*. Mol. Ecol. 23 (2), 421–434.
Kuester, A.P., Jones, R.W., Sappington, T.W., Kim, K.S., Barr, N.B., Roehrdanz, R.L., Senechal, P., Nason, J.D., 2012. Population structure and genetic diversity of the boll weevil (Coleoptera: Curculionidae) on *Gossypium* in North America. Ann. Entomol. Soc. Am. 105 (6), 902–916.
Kuroki, K., Kodama, T., 1987. Life history and control of the chestnut weevil, *Curculio dentipes* Roelofs, on the chestnut cultivate, ganne tree. Bulletin of the Yamaguchi Agricultural Experiment Station.
Lemic, D., Benítez, H.A., Püschel, T.A., Gašparić, H.V., Šatvar, M., Bažok, R., 2016. Ecological morphology of the sugar beet weevil Croatian populations: Evaluating the role of environmental conditions on body shape. Zoologischer Anzeiger-A J. Comp. Zool. 260, 25–32.
Marvaldi, A.E., Sequeira, A.S., O'Brien, C.W., Farrell, B.D., 2002. Molecular and morphological phylogenetics of weevils (Coleoptera, Curculionoidea): do niche shifts accompany diversification? Syst. Biol. 51 (5), 761–785.
Matsubayashi, K.W., Ohshima, I., Nosil, P., 2010. Ecological speciation in phytophagous insects. Entomol. Exp. Appl. 134 (1), 1–27.
McKenna, D.D., Sequeira, A.S., Marvaldi, A.E., Farrell, B.D., 2009. Temporal lags and overlap in the diversification of weevils and flowering plants. Proc. Natl. Acad. Sci. 106 (17), 7083–7088.
Miranda-Pérez, A., Castillo, G., Hernández-Cumplido, J., Valverde, P.L., Borbolla, M., Cruz, L.L., Tapia-López, R., Fornoni, J., Flores-Ortiz, C.M., Núñez-Farfán, J., 2016. Natural selection drives chemical resistance of *Datura stramonium*. PeerJ 4, e1898. http://dx.doi.org/10.7717/peerj.1898.
Mynhardt, G., 2006. Population genetics of the pecan weevil, Curculio caryae Horn (Coleoptera: Curculionidae), inferred from mitochondrial nucleotide data (Doctoral Dissertation, Texas A&M University).
Nakamine, H., Takeda, M., 2008. Molecular phylogenetic relationships of flightless beetles belonging to the genus *Mesechthistatus* Breuning, (Coleoptera: Cerambycidae) inferred from mitochondrial COI gene sequences. J. Insect Sci. 8 (1).
Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press.
Oberprieler, R.G., Marvaldi, A.E., Anderson, R.S., 2007. Weevils, weevils, weevils everywhere. Zootaxa 1668, 491–520.
Oliveira, D.C., Almeida, F.C., O'Grady, P.M., Armella, M.A., DeSalle, R., Etges, W.J., 2012. Monophyly, divergence times, and evolution of host plant use inferred from a revised phylogeny of the *Drosophila repleta* species group. Mol. Phylogenet. Evol. 64 (3), 533–544.
Pagel, M., 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst. Biol. 48 (3), 612–622.
Parra, L Mutis, Aguilera, A., Rebolledo, R., Quiroz, A., 2009. Estado del conocimiento sobre el cabrito del frambueso (cf), *Aegorhinus superciliosus* (Guérin) (Coleoptera: Curculionidae). Idesia 27 (1), 57–65.
Rambaut, A., Drummond, A.J., 2014. TreeAnnotator v2.1.2. University of Edinburgh, Institute of Evolutionary Biology, Edinburgh.
Rambaut, A., 2012. FigTree v1. 4. Molecular Evolution, Phylogenetics and Epidemiology. University of Edinburgh, Institute of Evolutionary Biology, Edinburgh, UK.
Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer.
Revell, L.J., 2012. phytools: a R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3 (2), 217–223.
Rohlf, F.J., 2004. TPS dig v. 1.4 Department of Ecology and Evolution, State University of New York, Stony Brook, NY. (http://life.bio.sunysb.edu/morph/).
Rozas, J., Sánchez-DelBarrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19 (18), 2496–2497.
Shankar, P., Kulkarni, V.M., Kumar, L.S., 2015. Male biased gene flow in banana pseudostem weevil (*Odoiporus longicollis* Oliver) as revealed by analysis of the COI-tRNALeu COII region. Genetica 143 (1), 85–92.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomological Soc. Am. 87 (6), 651–701.
Sota, T., Hayashi, M., Iwai, D., 2004. Phylogeography of the leaf beetle *Chrysolina virgata* in wetlands of Japan inferred from the distribution of mitochondrial haplotypes. Entomol. Sci. 7 (4), 381–388.
Suchard, M.A., Weiss, R.E., Sinsheimer, J.S., 2001. Bayesian selection of continuous-time Markov chain evolutionary models. Mol. Biol. Evol. 18 (6), 1001–1013.
Supple, M., Papa, R., Counterman, B., McMillan, W.O., 2014. The genomics of an adaptive radiation: insights across the Heliconius speciation continuum. In: Ecological Genomics. Springer, Netherlands, pp. 249–271.
Toju, H., Sota, T., 2009. Do arms races punctuate evolutionary stasis? Unified insights from phylogeny, phylogeography and microevolutionary processes. Mol. Ecol. 18 (18), 3940–3954.
Toju, H., 2009. Natural selection drives the fine-scale divergence of a coevolutionary arms race involving a long-mouthed weevil and its obligate host plant. BMC Evol. Biol. 9 (1), 273.
Wise, M.J., 2007. Evolutionary ecology of resistance to herbivory: an investigation of potential genetic constraints in the multiple-herbivore community of *Solanum carolinense*. New Phytol. 175 (4), 773–784.