SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Phylogeographical Analysis of Neotropical *Rhagoletis* (Diptera: Tephritidae): Did the Andes Uplift Contribute to Current Morphological Differences?

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Análisis Filogeográfico de *Rhagoletis* Neotropicales (Diptera: Tephritidae): ¿ha Contribuido el Levantamiento de los Andes a Explicar las Actuales Diferencias Morfológicas?

RESUMEN - Las especies de *Rhagoletis* neotropicales han sido agrupadas en cuatro grupos: *nova*, *psalida*, *striatella* y *ferruginea*, constituyendo 18 especies. Se han descrito diferencias morfológicas entre estas especies a ambos lados de la cordillera de los Andes que podrían relacionarse con el proceso de levantamiento cordillerano. En este trabajo se evalúa esta hipótesis usando análisis filogenético de atributos morfológicos y moleculares. Los resultados muestran que: a) las especies Neotropicales de *Rhagoletis* constituyen un grupo separado de las especies Palearticas y Norteamericanas, con la excepción de un miembro del grupo *striatella* el cual presenta cierta asociación con las especies Norteamericanas; 2) Las especies Neotropicales parece conformar un clado monofilético; 3) La separación de los grupos Sudamericanos de otros grupos fue estimada en 4.333 millones de años antes del presente, proceso anterior a la emergencia del puente de tierra entre América Central y Sudamérica; 4) Dentro de las especies con distribución Sudamericana, los caracteres morfológicos y moleculares coinciden en ubicar algunas especies del grupo *ferruginea* separadas de especies de *Rhagoletis* Neotropicales. 5) La separación del grupo *ferruginea* fue estimada en 3.882 millones de años antes del presente, evento que precede al último levantamiento de los Andes. La diversificación de los grupos *ferruginea*, *psalida* y *nova* a uno y otro lado de la cordillera de los Andes parece responder inicialmente a un proceso vicariante y posteriores eventos de dispersión y aislamiento. Estos resultados sugieren que el levantamiento de los Andes habría participado en los patrones de diversificación de las *Rhagoletis* Neotropicales.

PALABRAS CLAVE: Mosca de la fruta, diversificación, filogenia, Sudamérica

ABSTRACT - Neotropical *Rhagoletis* species are arranged in four groups: *nova*, *psalida*, *striatella* and *ferruginea*, which include 18 species. On both sides of the Andes, the evolution of morphological differences among these groups has been suggested to be related to the Andes uplift process. In order to test this hypothesis, a phylogenetic analysis of morphological and molecular data was performed. The results suggest that: 1) Neotropical species of *Rhagoletis* constitute a separate group from Paleartic and North American species, with the only exception being a member of the *striatella* group having a certain association with the northern species. 2) Neotropical species seem to form a monophyletic clade, although statistical support for this is weak. 3) The split of South American *Rhagoletis* from other groups was dated at 4.333 million years ago, which is before the emergence of a continuous landbridge between Central and South América. 4) Within species distributed in South América, morphological and molecular data were coincident, placing species of the *ferruginea* group separate from the other Neotropical *Rhagoletis*. 5) The divergence of the *ferruginea* group from the other groups was dated at 3.882 million years ago, which is before the last uplift of the Andes. These results suggest that diversification of the *ferruginea*, *psalida* and *nova* groups, on each side of the Andes, was the result of a vicariant separation followed by dispersal and isolation processes. Thus, these results support the hypothesis that the Andes uplift has played an important role in Neotropical *Rhagoletis* diversification.

KEY WORDS: Fruit fly, diversification, phylogeny, South América

The genus *Rhagoletis* Loew has been studied mainly because of its economic importance as an agricultural pest to fruits in many countries, and because it is considered a model of sympatric speciation processes. Within *Rhagoletis,* species with Neotropical distribution have been arranged into four groups: 1) the *nova* group (*R*. *penela* Foote, *R*. *nova* (Schiner), *R*. *conversa* (Brèthes), *R*. *tomatis* Foote, *R*. *lycopersella* Smyth, *R*. *willinki* Aczél and *Rhagoletis brncici* Frias); 2) the *psalida* group (*R*. *psalida* Hendel, *R*. *rhytida* Hendel and *R*. *metallica* Hendel); 3) the *striatella* group (*R*. *striatella* Wulp, R. *macquartii* (Loew), *R*. *jamicensis* Foote, *R*. *triangularis* Hernández & Frías, *R*. *solanophaga* Hernández & Frías and *R*. *nicaraguensis* Hernández & Frías); and 4) the *ferruginea* group (*R*. *ferruginea* Hendel, *R*. *adusta* Foote and *R*. *blanchardi* Aczél) (*sensu* Foote 1981, Hernández-Ortiz & Frías 1999). Although there is little information regarding host affiliation, most of these species are mainly associated with the Solanaceae (Bush 1966, Foote 1981, Frías 1992). Members of the South American genus *Rhagoletis* were described to form a coherent monophyletic group, although their relationships are still a matter of study (Smith *et al*. 2006).

Morphological differences among Neotropical *Rhagoletis* distributed east and west of the Andes have been described (Frías 1992). All *Rhagoletis* species located east of the Andes (with the exception of *R*. *willinki*) contain a complete posterior apical crossband which extends from the sub-apical band to the wing margin, whereas species located west of the Andes lack this band or have it present as an elongated spot (Fig. 1). Similarly, species east of the Andes have a spherical spermatheca, which differs from those having a rodlike spermatheca on the west side of the Andes (Fig. 1). Therefore, it is likely that the Andes uplift may have played a certain role in the diversification of Neotropical *Rhagoletis*, although no independent evidence has corroborated this. It is worth noting that the Andes have played some role in the generation of different races of the butterfly *Heliconius erato* L., where a basal split between groups of races from the east and west of the Andes seems to have occurred at the beginning of the Pleistocene by vicariant separation (Brower 1994). A similar process could have taken place in Neotropical *Rhagoletis* species.

The aim of this study was to examine the phylogeography (*sensu* Avise 2000) of Neotropical *Rhagoletis* species. In particular, the aim was to test the hypothesis regarding the possible role of the Andes uplift as a diversifying mechanism. First, phylogenetic affinities among Neotropical *Rhagoletis* were studied based on a set of morphological data. Second,

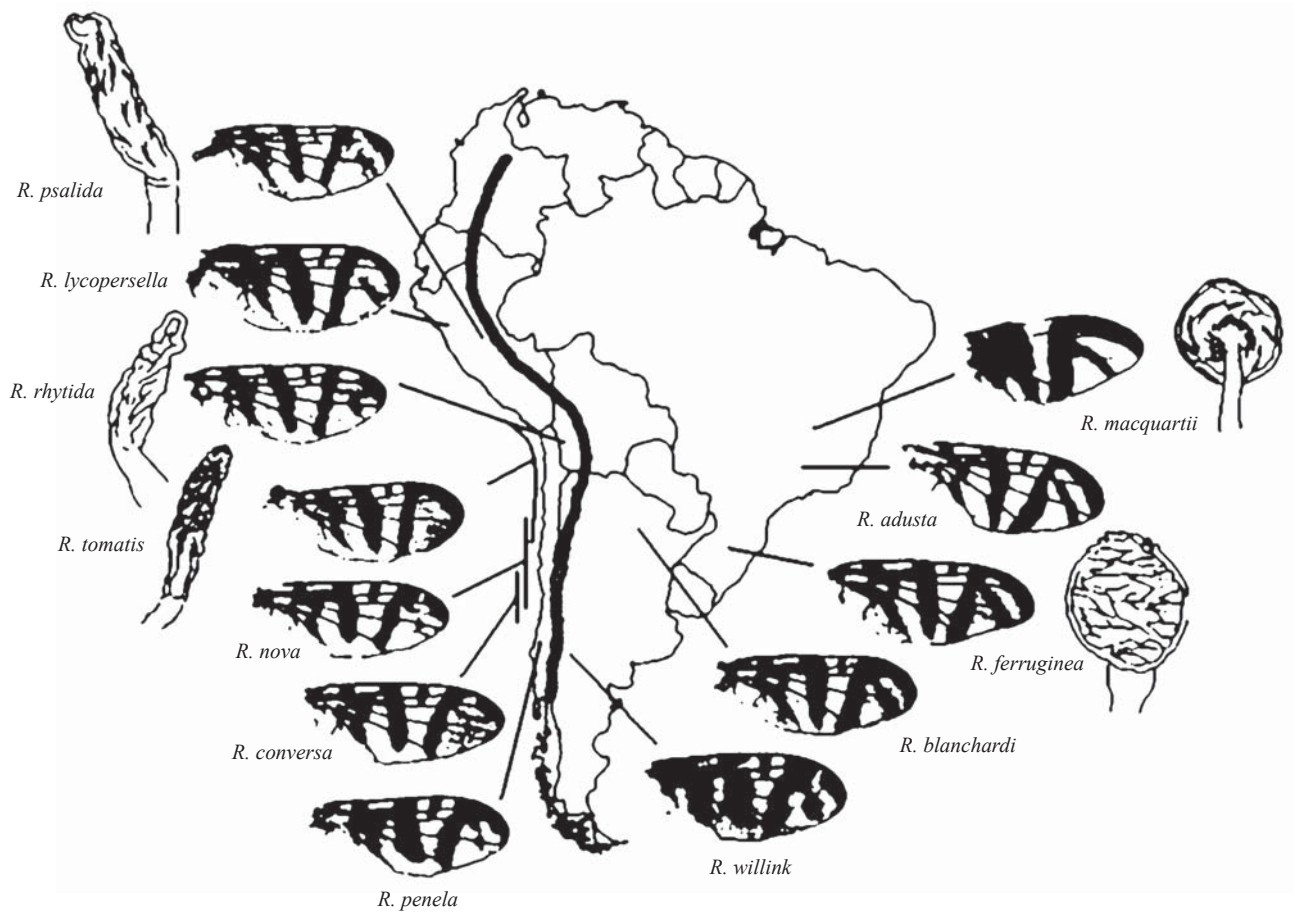

Fig. 1. Diagram showing differences in wind pattern and spermatheca shape of Neotropical *Rhagoletis* species. The broad line crossing the map of South America represents the Andes peaks. Main locations of records are indicated for each species with a thin line.

a phylogenetic analysis with estimations of divergence times of clades based on available mitochondrial cytochrome c oxidase subunit II (COII) DNA sequences was performed. In both cases, sequences of North American and Palearctic *Rhagoletis* species were also included in order to study their relationship with the Neotropical species. Finally, a dendrogram based on the similarity of their geographical ranges (biogeogram) was built to estimate the geographic relationship of the Neotropical *Rhagoletis* species

Material and Methods

Molecular data. We examined available COII sequences from sixteen species and obtained new sequences for two more species (Table 1). The outgroup species *Ceratitis capitata* (Wiedemann) was chosen on its taxonomic position within the Tephritidae (Smith & Bush 1997, McPheron & Han 1997). New sequences for *R. nova* and *R. tomatis* were obtained by isolating DNA and following an optimized protocol for alcohol-preservation of individual flies (Han & McPheron 1997). The primers used for PCR amplification were: CITOX-5: 5'ATG-ACA-ACA-TGA-GCT-GCC-CTT-GGC-CTT-CAA3' (forward) and CITOX-3: 5'TGA-ATT-TAC-TCT-ATT-TTT-AAT-TCA-TTT-AAT3' (reverse) (Gasparich *et al*. 1995). PCR was performed in 25 µl reaction volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 6.7 mM MgCl2, 0.25 mM each of dATP, dCTP, dGTP and dTTP, 0.8 µM of each primer, 1.5 units of Taq polymerase (Life Technologies) and 25 to 100 ng of template DNA. After the initial denaturation (95°C, 3 min), PCR was run for 40 cycles consisting of a 94°C denaturation step (1 min), a 48°C annealing step (1 min) and a 72°C elongation step (2 min) in a MJ Research, PTC200 thermocycler. At the end of the run, a final cycle of 94°C (1 min), 50°C (1 min) and 72°C (9 min) was carried out. Finally, samples were maintained at 4°C and stored at -20°C until digestions or cloning could be performed. Five µl aliquots of each PCR product were loaded onto a 1.5% agarose gel in TAE buffer (Sambrook *et al*. 1989), and electrophoresis was carried out at 7 volts cm µ1. Amplified products were visualized under UV using ethidium bromide. The sizes of the resulting DNA fragments were estimated by comparison with commercial size markers (100 bp and 1 kbp DNA ladders, Life Technologies, USA). Bands were excised from gels and DNA was extracted using a Wizard PCR purification kit (Promega, USA). Purified PCR products were ligated into the pGEM5-T cloning vector (Promega, USA) as described by Salazar *et al*. 2002. DNA sequencing of the inserts in pGEM5-T was performed by the Departamento de Ciencias Ecológicas, Pontificia Universidad Católica de Chile. Sequencing data for the COII sequence could not be obtained for *R. penela* because of technical difficulties.

Distribution of the species belonging to either side of the Andes was based on records as appeared in Foote (1981). Highest mountain peaks describe an imaginary line dividing the Andes in two sides to which species can be assigned to (Fig. 1). A special case involved the specie *R . psalida* and *R.*

Table 1. List of taxa examined with geographic origin and Genbank accession number for cytochrome oxidase subunit II (COII) mitochondrial gene.

Species	Distribution	GenBank accession number COII
R. nova	Neotropical (western Andes)	AF228051[1]
R. tomatis	Neotropical (western Andes)	AF228050[1]
R. conversa	Neotropical (western Andes)	U53263[2]
R. lycopersella	Neotropical (western Andes)	AY310724[2]
R. psalida	Neotropical (western Andes)	AY310727[2]
R. blanchardi	Neotropical (eastern Andes)	AY310720[2]
R. ferruginea	Neotropical (eastern Andes)	AY310721[2]
R. striatella	Neotropical, North American (eastern Andes)	U53262[2]
R. ribicola	North American	U53246[2]
R. suavis	North American	U53252[2]
R. cingulata	North American	U53248[2]
R. tabellaria	North American	U53240[2]
R. pomonella	North American	U53229[2]
R. cerasi	Paleartic	U53257[2]
R. basiola	Paleartic	U53261[2]
R. alternata	Paleartic	U53260[2]
R. barberides	Paleartic	U53258[2]
C. capitata	Neotropical, North American, Paleartic	CCU53270

[1]Salazar *et al*. 2002; [2]Smith *et al*. 1999.

rhytida of the *psalida* group, with distribution in the higher Altiplano, but no records and the east of Andes.

Morphological data. A total of 19 characteristics based on adult external morphology were obtained from the literature (Table 2). Character states were recorded as absent (0) or present (1), and also as multistate (e.g. 0, 1, 2). Eighteen *Rhagoletis* species plus the outgroup species *C. capitata* were included. Main sources of information used here were the studies of Foote (1981), Hernández-Ortiz & Frías (1999) and the database of Carroll *et al*. (2005).

Phylogenetic analyses of morphologcal data. Phylogeny reconstruction was performed using maximum parsimony as implemented by PAUP (version 4.0b10, Swofford 2002). Hierarchical structure in the data was assessed by partitioning tail permutation test (PTP test; Faith & Cranston 1991) as implemented in PAUP. Parsimonious trees were found through a heuristic search strategy using TBR branch swapping. Branch support was based on calculating bootstrap values (10.000 replicates and with rearrangement limit of 10.000.000 rearrangements per addition sequence) and by estimating Bremer decay values using Autodecay 5.0 (Eriksson 2001).

Phylogenetic analyses of molecular data. COII sequences were aligned with ClustalW (Gibson *et al*. 1996). Sequences were analyzed as unordered, excluding those which were phylogenetically uninformative. Partitioning tail permutation (PTP; Faith & Cranston 1991) as implemented in PAUP was used to evaluate non-random hierarchical structure in the data (heuristic searches with 1.000 replicates).

A COII phylogenetic hypothesis was generated using neighbour-joining tree (NJ), maximum parsimony (MP) and maximum-likelihood (ML) analysis. These approaches were chosen because best phylogenetic hypotheses are most likely to be reliable when congruent evidences are obtained independently (Barkman *et al*. 2000). Neighbour-joining analysis (Saitou & Nei 1987) was conducted using the Tajima-Nei distance to account for the nucleotide bias in animal mtDNA (Tajima & Nei 1984), implemented in MEGA3 (Kumar *et al*. 2004). Maximum parsimony analysis

Table 2. Data matrix used to construct phylogeny based on morphological traits.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*C. capitata*	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
2	*R. lycopersela*	1	0	2	1	2	0	1	1	0	1	1	1	0	1	1	0	1	0	1
3	*R. tomatis*	1	1	2	1	2	0	1	1	0	0	1	1	0	1	1	0	1	1	1
4	*R. nova*	1	2	2	1	2	0	1	0	0	0	1	1	1	1	1	0	1	1	1
5	*R. conversa*	1	0	2	1	2	0	1	0	0	0	1	1	1	1	1	0	1	1	1
6	*R. willinki*	1	1	2	0	2	0	0	0	1	1	1	1	1	1	1	0	1	1	1
7	*R. penela*	1	1	2	0	2	0	0	1	0	1	1	1	1	1	1	0	1	1	1
8	*R. psalida*	0	0	0	2	1	0	1	1	0	1	1	1	0	1	1	0	1	1	1
9	*R. rhytida*	0	0	0	3	1	0	1	0	0	1	1	1	0	1	1	0	1	1	1
10	*R. metallica*	0	0	0	0	1	0	1	0	0	1	0	1	0	1	1	0	1	1	1
11	*R. striatella*	1	0	1	3	1	1	1	1	0	2	0	0	0	1	1	1	0	1	1
12	*R. macquartii*	1	0	2	3	3	0	0	1	1	2	1	0	0	1	1	1	?	2	1
13	*R. jamicensis*	1	0	2	3	1	0	1	1	1	2	1	0	0	1	1	1	?	0	1
14	*R. triangularis*	0	0	0	1	1	0	1	1	0	2	1	0	0	1	1	?	0	1	1
15	*R. nicaraguensis*	1	2	2	2	1	1	1	1	0	2	1	0	0	1	1	?	?	0	1
16	*R. solanophaga*	1	1	2	2	1	1	1	1	0	2	1	0	0	1	0	1	1	0	1
17	*R. ferruginea*	1	0	2	1	2	0	1	0	1	2	1	1	0	0	0	1	0	1	2
18	*R. adusta*	1	0	2	1	2	0	1	1	1	2	1	1	0	0	0	1	0	1	2
19	*R. blanchardi*	1	1	2	1	2	0	1	1	1	2	1	1	0	0	0	?	0	1	2

1) Scutun bars (1 = present; 0 = absent). 2) Bars in scutal united (0 = none united; 1 = two on each side united; 2 = all united). 3) Number of scutal bars (0 = 0; 1 = 2; 2 = 4). 4) Abdominal tergites with marginal fasciae (0 = none; 1 = yellow; 2 = white; 3 = yellow to white). 5) Transverse wing bands (1 = 3; 2 = 4). 6) Discal and sub-apical band unites (1 = present; 0 = absent). 7) Sub-basal and discal bands apart (1 = apart; 0 = close). 8) Anterior apical band complete (1 = complete; 0 = not complete). 9) Anterior separated from costal (hyaline space) (1 = present; 0 = absent) 10) Posterior Apical band (0 = absent; 1 = spot, 2 = band).11) Anterior apical attached to sub-apical accessory (1 = attached; 0 = not attached). 12) costal bar (1 = present; 0 = absent). 13) Ovipositor with subapical lateral projection (1 = present; 0 = absent). 14) Abdomen predominantly black (1 = present; 0 = absent). 15) Thorax color black (1 = present; 0 = absent). 16) Spermateca spherical (1 = present; 0 = absent). 17) Distiphallus with spinelike setulae (1 = present; 0 = absent). 18) Third antenna segment (0 = rounded; 1 = pointed; 2 = curved). 19) Predominant body colour (0 = yellow; 1 = black; 2 = brown)

to find equally parsimonious trees was performed using heuristic search with TBR branch swapping with PAUP. For ML analysis, Modeltest version 3.04 (Posada & Crandall 1998) with the Akaike information criterion (AIC) was used to select an appropriate nucleotide substitution model. The best model fitting the data was the GTR+G+ I. Thus, ML was further performed with a heuristic search strategy using TBR branch swapping in PAUP.

Branch support was accomplished by calculating non-parametric bootstrap values. Support for NJ analysis was performed with 10.000 replicates. For MP analysis, 10.000 replicates using branch-swapping by TBR and 10 random addition sequence replicates were performed. For ML, 500 replicates with initial tree were obtained via random addition, also using branch-swapping by TBR with GTR+G+I substitution model. Support for branches in MP analysis was also investigated by extracting Bremer decay values which were computed with Autodecay 5.0 (Eriksson 2001) and the PAUP search parameters: *hsearch addseq = random nreps = 100*. For all topologies, confidence values lower than 50% are not shown.

A Bayesian analysis of molecular data was carried out to estimate the time of divergence of the Neotropical *Rhagoletis* by using the software BEAST v1.4.6 (Drummond and Rambaut, 2003). Two types of analyses were preformed: one using a known constant mutation rate (strict clock) and other with an unknown mutation rate (relaxed clock). For the strict clock analysis, the COII mean substitution rate per million years was set to a value of 2.1 following the estimation provided by Brower (1994). Both analyses were preformed either with or without prior information of a previously reported divergence time of a given clade. In the case where dating was included, Yule process of speciation according to the time of divergence of *R. pomonella* from a Mexican ancestor, which has been estimated at 1.57 million years ago (Feder *et al*. 2003), was included. Analyses included five independent runs of MCMC for 1.0×10^6 steps sampling every 1000 under the GTR model. Estimations of divergence times and their corresponding errors (lower and upper 95% highest posterior densities) were viewed using the software Tracer v1.1.1 (Drummond & Rambaut 2003). To obtain a maximum clade credibility tree, trees were summarized with the software TreeAnnotator 1.4.6 (http://tree.bio.ed.ac.uk/software/treeannotator/), and the final tree topology was viewed with FigTree v.1.1.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Geographic relationship of the Neotropical *Rhagoletis* species. To estimate the geographic relationship of Neotropical *Rhagoletis* species with distribution in South América, a dendrogram based on the similarity of their geographical ranges (a biogeogram) was constructed. Distribution ranges for *Rhagoletis* species were obtained from the literature, mainly from the studies of Foote (1981) and Hernández-Ortiz and Frías (1999). A map of South América (1:400 km) was divided into squares of 1.5° longitude and latitude, and each square was numbered. This map was overlayed on a map of the distribution of each species, and the number of squares in which each species was present was recorded. Thus, a matrix of Euclidean distances between *Rhagoletis* species was constructed on the basis of their presence or absence in each square. The dendrogram was constructed using cluster analysis of this matrix using Ward's Method implemented in SPSS statistical software.

Results

Morphological phylogeny. Significant value of PTP test ($P < 0.01$) suggests the presence of a phylogenetic signal in morphological data, supporting further analysis. All characters were parsimony-informative. The heuristic search for a maximum parsimony solution resulted in only one parsimonious tree (length = 56, HI= 0.518, CI = 0.482, RI = 0.663, excluding uninformative characters; Fig. 2). This tree

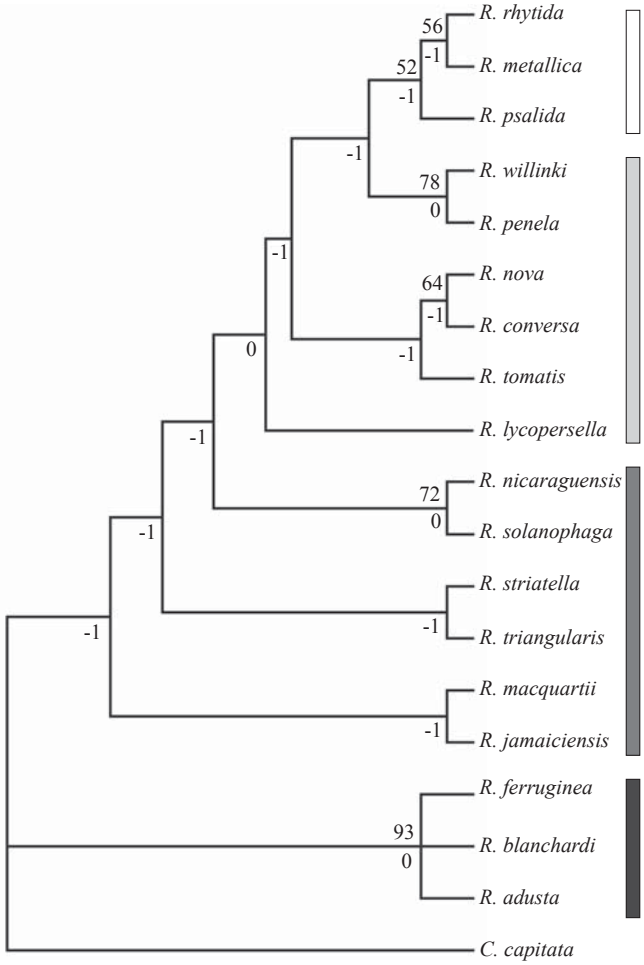

Fig. 2. Maximum-parsimony single consensus trees for 19 *Rhagoletis* species inferred from 19 morphological characters. Numbers above the branches are bootstrap values (≥ 50% majority rules; 10000 replicates), below are decay indices. Unlabelled branches are considered unresolved using these methods. Vertical filled bars indicate the group to which each species belongs: ■: *ferruginea* group; ■: *striatella* group; ■: *nova* group; □: *psalida* group.

contained 15 resolved nodes of which many were strongly supported by decay values and in some nodes bootstrap values showed more resolution

Molecular phylogeny. The significant value of PTP test ($P < 0.01$) indicates that COII sequence data have a cladistic structure. This gene sequence resulted in 687 nucleotides in all *Rhagoletis* including the outgroup *C. capitata*. Of the 687 characters obtained for COII sequences, 253 (36.8%) were variable, with 159 of these being phylogenetically informative, and 48 at first or second codon positions. The base composition was not different across taxa ($\chi^2 = 18.7$ (d.f.=51), $P = 0.99$). Fig. 3 shows frequencies of transversions and transitions for COII, indicating a bias for transitions (ti/tv = 3.78).

Neighbour-joining tree (Fig. 4), maximum parsimony (Fig. 5), maximum-likelihood analysis (Fig. 6) and Bayesian analysis using a strict clock (Fig. 7) of COII resulted in rather similar topologies. MP analysis of the aligned data set yielded up to five parsimonious trees (length = 590, HI = 0.459, CI = 0.540, RI = 0.498, excluding uninformative characters; Fig. 5 shows the strict consensus tree). ML analysis resulted in five best trees with a natural log likelihood score of -3596.69. This tree was similar in topology to the maximum parsimony tree, except for the position of *R. cerasi* and for the node separating the species of the *ferruginea* group from those of the *nova* group. Unlike the other analyses, the Bayesian phylogeny placed *R. tomatis* as the ancestor of *nova* and *psalida* groups.

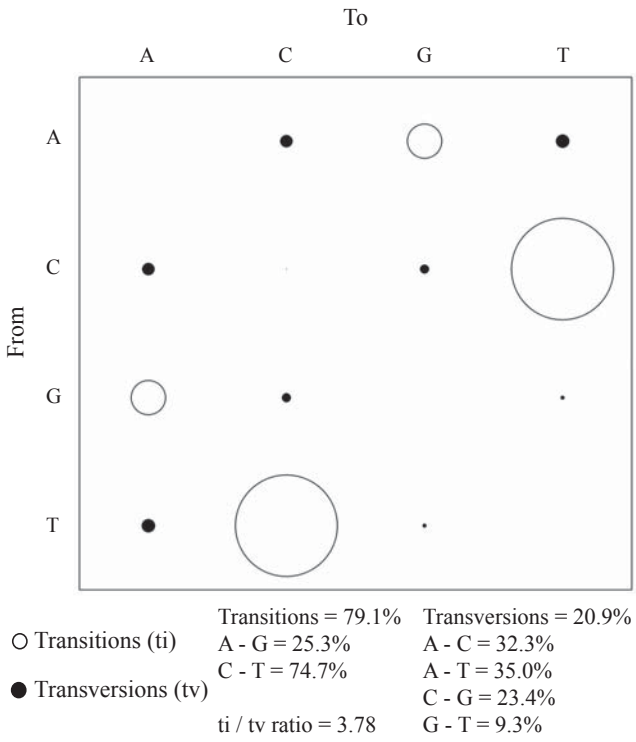

Fig. 3. Diagram showing the frequency and percentage of substitution types (transitions and transvertions) for COII sequences of *Rhagoletis* species analysed in the present study.

Genetic divergence. The pattern of sequence divergence estimated with Beast is shown in Fig. 7. The times of divergence estimated with strict and relaxed clock were very similar, either with or without calibrating using a known divergence time. For instance, the split between the South American and North American *Rhagoletis* was dated at 4.333 Mya (with lower and upper 95% highest posterior densities of 3.837 and 4.836 Mya, respectively; estimated as a strict

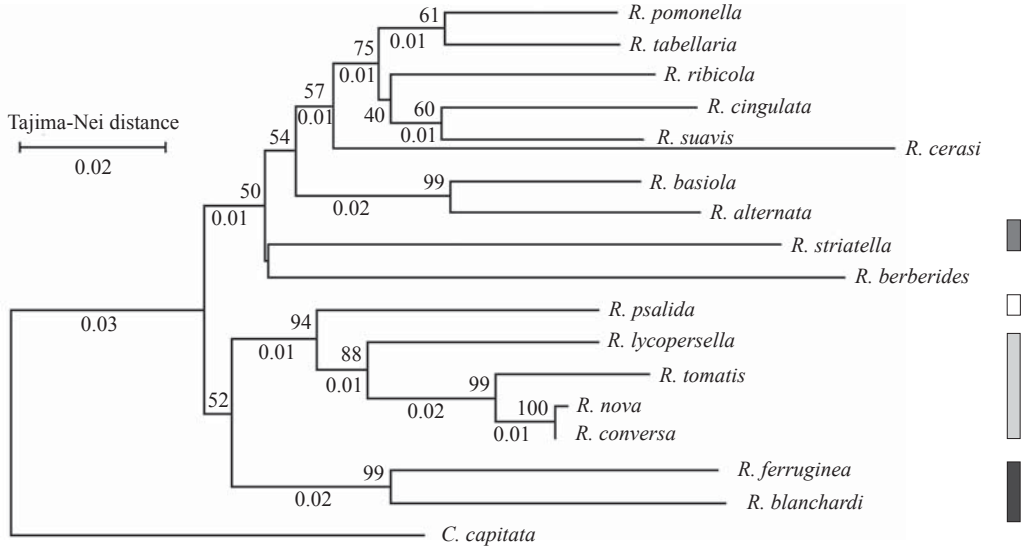

Fig. 4. Single topology obtained using neighbour-joining analysis, based on Tajima-Nei distance for mtDNA COII of *Rhgoletis* species. Numbers above the branches are bootstrap values (≥ 50% majority rules; 500 replicates), below are percentages of nucleotide divergence for each node (obtained from the 2.1% per million years rate). Vertical filled bars indicate the group to which each species belongs: ■: *ferruginea* group; ■: *striatella* group; ■: *nova* group; □: *psalida* group.

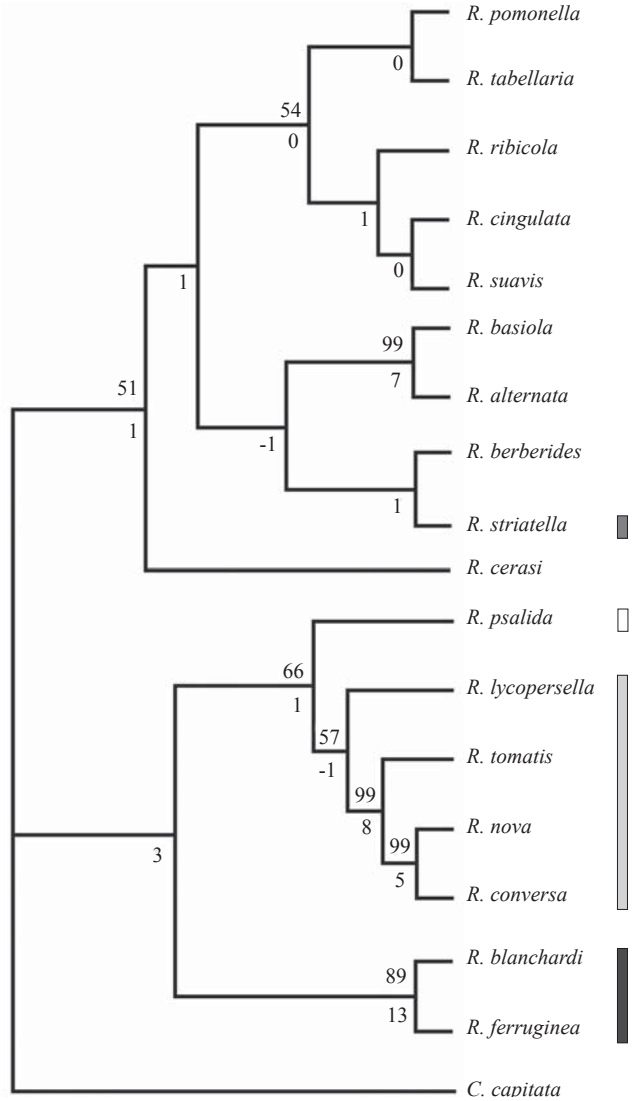

Fig. 5. Strict consensus tree out of the five most parsimonious trees for 17 *Rhagoletis* species based on mtDNA COII sequence. Numbers above the branches are bootstrap values (≥ 50%), below are decay indices. Vertical filled bars indicate the group to which each species belongs: ■: *ferruginea* group; ■: *striatella* group; ■: *nova* group; □: *psalida* group.

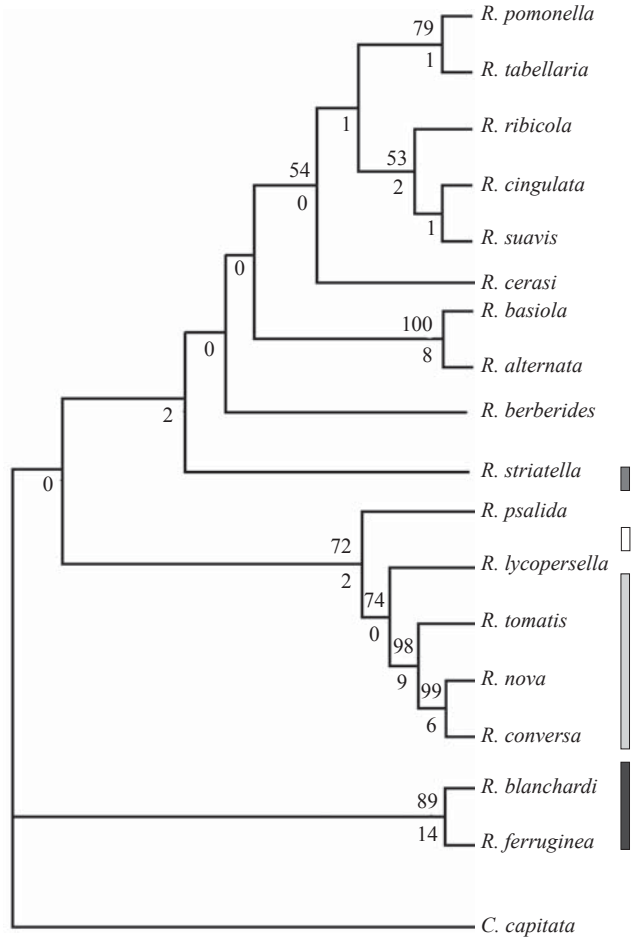

Fig. 6. Single maximum likelihood tree for Neotropical *Rhagoletis* species relationships, based on mtDNA COII, using the GTR-G-I model. Numbers above the branches are bootstrap values (≥ 50%), below are decay indices. Vertical filled bars indicate the group to which each species belongs: ■: *ferruginea* group; ■: *striatella* group; ■: *nova* group; □: *psalida* group.

clock without priors). The split between the *ferruginea* group and the *nova* and *psalida* groups was estimated to occur at 3.882 Mya (with lower and upper 95% HPD of 3.417 and 4.376 Mya). Within the *ferruginea* group, the split between *R. ferruginea* and *R. blanchardi* was dated to occur 1.917 Mya (with lower and upper 95% HPD of 1.437 and 2.393 Mya). In the case of the *psalida* and *nova* groups, divergence of estimation overlapped to a large extent, with *R. tomatis* diverging from the other species 1.85 Mya (with lower and upper 95% HPD of 1.467 and 2.22 Mya), followed by the divergence of *R. psalida* from the rest of the species occurring 1.772 Mya (with lower and upper 95% HPD of 1.41 and 2.129 Mya) and the divergence of *R. lycopersella* occurring 1.643 My ago (with lower and upper 95% HPD of 1.275 and 2.015 Mya). Within this group, the most recent divergence was dated for the species *R. nova* and *R. conversa*, estimated to have occurred only 75,210 year ago (with lower and upper 95% HPD of 2.580 and 178,600 years ago).

Geographic relationship. Cluster analysis with Ward's method identified six main groups of *Rhagoletis* species according to the degree of similarity of their geographical ranges (Fig. 7). Most of the *Rhagoletis* species with east Andes distribution were grouped together (top cluster in Fig. 7). These species belonged to a wide range of groups including *psalida*, *striatella*, *ferruginea* and *nova* groups. Contrastingly, species with distribution to the west of the Andes were joined in another cluster (bottom cluster in Fig.7), clusters that included only species of *nova* group. Of this last group, only *R. willinki* has been recorded at the east of the Andes.

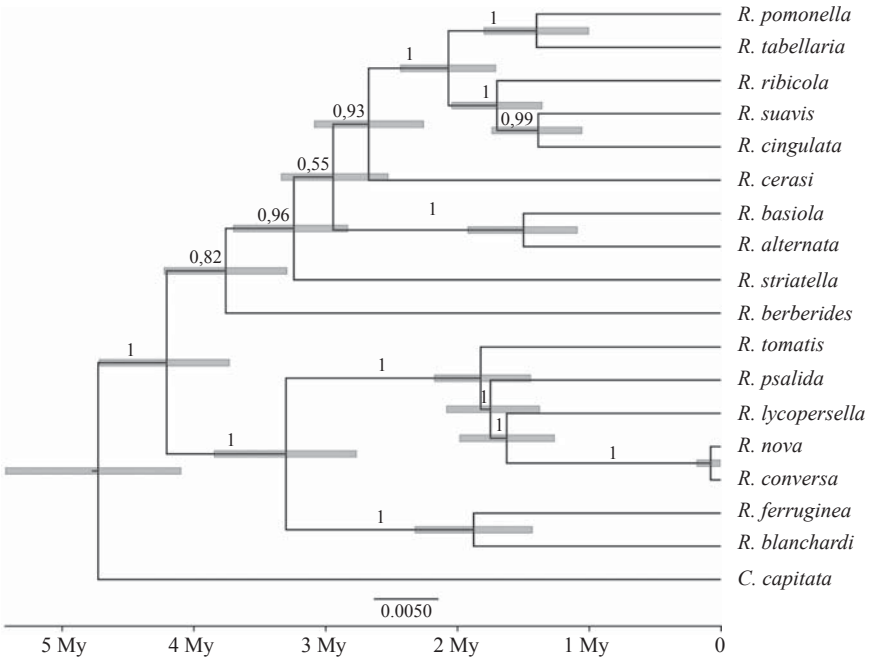

Fig.7. Maximum clade credibility tree for Neotropical *Rhagoletis* species relationships based on mtDNA COII, using Bayesian MCMC analysis of molecular sequences. The tree was built under the strict clock model without setting priors. Bars represent the 95% highest posterior density (HPD) intervals for the divergence time estimates. The divergence times correspond to the mean posterior estimate of their age in millions of years. Each node in the tree is labelled with its posterior probability if it is greater than 0.5.

Discussion

Molecular data suggest that the Neotropical *Rhagoletis* formed a single lineage, apart from the North American and the Paleartic *Rhagoletis* species, although the resolution was poor. For instance, NJ, MP, and ML did not verify bootstrap or Bremer support for separating both groups. A similarly low resolution was found in a previous phylogeny for the North American *Rhagoletis* with the same sequence, which only included two Neotropical species (Smith & Bush 1997). Using the 16S ribosomal RNA gene, McPheron & Han (1997), also found low resolution for the position of the Neotropical species therein (*R. striatella* and *R. conversa*). As mentioned by Smith & Bush (1997), mitochondrial COII data seems insufficient to resolve these distant relationships. Nevertheless, the present analysis, with the inclusion of the other six Neotropical *Rhagoletis* species, was able to establish relationships between the Neotropical *Rhagoletis* groups. Bayesian estimation of phylogenetic relationships found better support for the separation of Neotropical *Rhagoletis* from other relatives (Fig. 7).

The morphological analysis suggests that the species of *striatella* group are basal to the *nova* and the *psalida* species, with no support by the bootstrap and Bremer values (Fig. 2). Differently, NJ, MP, ML, and Bayesian analysis for the molecular data found that *R. striatella* showed more affinities with the Paleartic *Rhagoletis* than with species from a southern distribution, although its position within the Paleartic *Rhagoletis* was also variable depending on the analysis performed. NJ and MP placed *R. striatella* with *R. berberidis* and separated them from the other Paleartic *Rhagoletis*, while ML and Bayesian analysis placed *R. striatella* at the most basal position relative to the North American and the Paleartic species, although this was not well supported by the bootstrap values. In any case, *R. striatella* never joined with the Neotropical *Rhagoletis*. This is consistent with the fact that *R. striatella* has the most northern distribution within the *striatella* group, which is also one of the more widely distributed groups in continental América (Hernández-Ortiz & Frías 1999).

Host-affiliation information for this group is quite poor, though it is known that larvae of *R. striatella* can develop in fruits of *Physalis* species (Solanaceae). All other species of the *striatella* group, and also all other Neotropical *Rhagoletis* species, breed mainly on Solanaceae (Foote 1981). These findings suggest that, despite the host affinities of the *striatella* group with groups distributing in South America, this group seems to have more affinity for the Paleartic and the North American species. Distribution of *R. macquartii* of the *striatella* group in South América may have been the result of an invasion and later speciation of a *striatella* ancestor that dispersed from Central América to South América. Thus, considering *striatella* as a separated group from the South American groups, the mitochondrial COII data suggest that the *ferruginea*, *psalida* and *nova* groups form a monophyletic clade. Monophylia of the South American *Rhagoletis* is very apparent in NJ and MP, but not in ML analysis, with only slight bootstrap support

in NJ analysis. Molecular analysis of Carpomyina subtribe performed by Smith *et al*. (2006) also found that South American *Rhagoletis* forms a monophyletic group. It is worth noting that in our analysis using a strict clock without setting prior information of a known divergence time for a clade, the split of *R. pomonella* and *R. tabellaria* was found to have occurred 1.398 Mya (with lower and upper 95% HPD of 0.9854 and 1.806 Mya, respectively), which is the range where divergence of *R. pomonella* from its Mexican ancestor was estimated (1.57 Mya) by Feder *et al*. (2003).

The split between the North American and Neotropical *Rhagoletis* was dated at about 4.333 Mya. The lower limit of confidence of this estimation is about the time of the emergence of a continuous landbridge between Central and South América, which occurred in the Pliocene about 3.5 Mya (Keigwin 1982). Considering that North American species, including the *striatella* group, possess more derived morphological features with respect to homologous structure in the Neotropical taxa (Bush 1966, Smith & Bush 1997), diversification of North American *Rhagoletis* species presumably occurred from a northern colonization/dispersion of a southern taxa throughout the existing land connection. Given that the only Neotropical taxon having an affinity with North American species was *R. striatella*, it is likely that one extinct dispersing taxon from *striatella* group was the ancestor of North American groups. Resolving phylogenetic relationships between all *striatella* group species and their affinities with North and South American species would contrast this hypothesis.

Morphological and molecular data were coincident, placing species of the group *ferruginea* separating from other Neotropical *Rhagoletis* (Fig 2, 4, 5, 6 and 7). Biogeogram also showed a geographical association between *ferruginea* group by placing together *R. blanchardi* and *R. ferruginea* separated from species with distributions to the west of the Andes (Fig. 8). Since the last uplift of the Andes occurred in the Plio-Pleistocene period (about 5 to 1.8 million Mya), and the split between *ferruginea* groups and other *Rhagoletis* groups occurred 3.882 Mya, it seems reasonable that this was due to a vicariant process elicited by the Andes uplift, separating species between those currently distributed to the east and west of the Andes. In the case of those species with distributions to the west side of the Andes, molecular data (NJ, MP and ML analysis) placed *R. psalida* in a basal position in relation to the *nova* species, which suggest that *nova* species originated from a *psalida* member. Smith *et al*. (2006) also found similar relationships when studying Carpomyina subtribe, demonstrating that analysis of DNA sequences of alleles at an anonymous nuclear locus indicated that *R. psalida* was also a sister to *R. conversa* and *R. nova*, with *R. lycopersella* being the sister taxon to this clade. On the other hand, since *psalida* species distribution currently overlaps with *R. lycopersella* (biogeogram in Fig. 8), which is the species of the *nova* group with a more northern distribution, it is likely that a primitive *psalida* member gave rise to *R. lycopersella* by sympatric speciation. Thereafter, a population of *R. lycopersella* may have experienced southern expansion, evolving into *R. tomatis*.

Bayesian analysis produced a different picture (Fig. 7). In this last case, *R. tomatis* is an ancestor of *R. psalida*, which is an ancestor of *R. lycopersella*, which in turn may have originated from *R. nova* and *R. conversa*. In

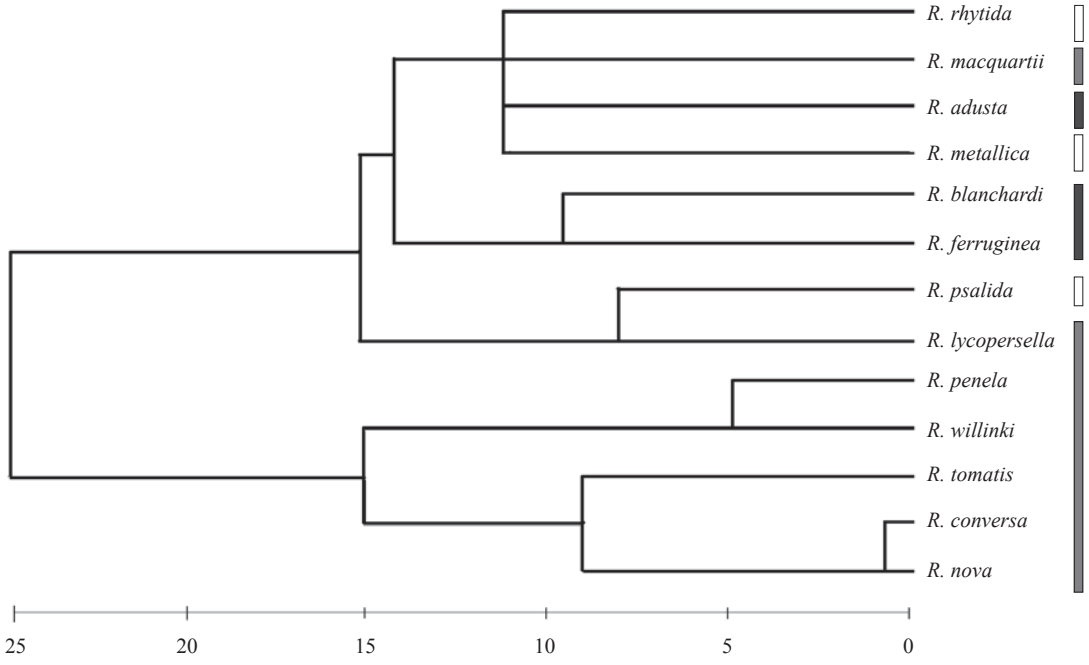

Fig. 8. Dendrogram of *Rhagoletis* species with a South American distribution on the basis of similarity of geographical ranges (biogeogram). Vertical filled bars indicate the group to which each species belongs: ■: *ferruginea* group; ■: *striatella* group; ■: *nova* group; □: *psalida* group.

addition, the climatic and biogeographic changes occurring as a consequence of the loss of continuity of subtropical vegetation in South América (Villagrán & Hinojosa 1997) may have caused the isolation of southern populations of *R. tomatis* or *R. lycopersella,* which subsequently may have given rise to the rest of the *nova* species. The present overlapping distribution of *R. nova* and *R. conversa* (Fig. 8), and the close phylogenetic affinity found herein, support the notion that these species have undergone a recent divergence. Because different host plants have been described for these species, namely *Solanum muricatum* (Aiton) for *R. nova,* and *Physalis* sp., *Solanum tomatillo* and *Solanum nigrum* for *R. conversa* (Foote 1981; Frías 1992, 2001; Salazar *et al*. 2002), a process of sympatric speciation by host shift could have promoted this divergence.

The lack of COII sequence data for *R. penela* and *R. willinki* specimens did not allow us to establish their phylogenetic relationship with other *nova* species. However, morphological and biogeographic data clearly show that *R. penela* and *R. willinki* are closely related. Frías (1992) suggested that *R. willinki* originated by dispersal of a population of *R. conversa*, a process that could have been facilitated by the low altitude of Andes at the latitude of Neuquen, where *R. willinki* are distributed. The present study confirms the close association between these species.

Our results support the notion that the Andes have played an important role during Neotropical *Rhagoletis* diversification. A vicariant process, promoted by the Andes uplift during the Pleistocene, was presumably followed by a succession of dispersal and isolation events during the Holocene that affected the divergence pattern on both sides of the Andes.

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