BIOTAXONOMY OF TEPHRITOIDEA
Isr. J. Entomol. Vol. 35-36, 2005/6, pp. 317-340

Analysis of Mitochondrial DNA and Morphological Characters in the Subtribe Carpomyina (Diptera: Tephritidae)

James J. Smith,[1] Matthew Jaycox,[1,2] Martha R.B. Smith-Caldas,[1,3] Guy L. Bush[1,4]

ABSTRACT

The phylogenetic relationships of 43 species in the subtribe Carpomyina (39 *Rhagoletis* spp., plus *Carpomya schineri* (Loew), *Oedicarena latifrons* (Wulp), *Rhagoletotrypeta pastranai* Aczél, and *Zonosemata electa* (Say)) are examined using morphological and mitochondrial DNA (mtDNA) characters. The taxon sample includes 5 Palearctic *Rhagoletis* species (*R. almatensis* Rohdendorf, *R. batava* Hering, *R. flavicincta* (Loew), *R. flavigenualis* Hering, and *R. magniterebra* (Rohdendorf)) and 5 Neotropical *Rhagoletis* species (*R. blanchardi* Aczél, *R. ferruginea* Hendel, *R. lycopersella* Smyth, *R. nova* (Schiner), and *R. psalida* Hendel) whose mtDNA relationships have not been previously analyzed. Phylogenetic analysis of 77 morphological features using unweighted parsimony yielded 28,671 most parsimonious reconstructions (MPRs). A strict consensus of these MPRs contained 12 clades, and further analysis using successive approximations improved phylogenetic resolution.

[1]Department of Zoology, Michigan State University, East Lansing, MI 48824-1115, USA. E-mail: jimsmith@msu.edu. [2]E-mail: jaycoxma@msu.edu. [3]Present address: Universidade Estadual de Santa Cruz, Departamento de Ciências Biológicas, Km 16, Rodovia Ilhéus-Itabuna, Ilhéus-Bahia-Brazil 45.650-000, BRAZIL. E-mail: msbressan@hotmail.com. [4]E-mail: bushfly@msu.edu

Analysis of 1027 aligned nucleotide positions in the mitochondrial COI/COII region indicated that, as previously hypothesized, the genus *Rhagoletis* may not be strictly monophyletic. Monophyly of *Rhagoletis*, in the strict holophyletic sense (*sensu* Hennig), was disrupted by *Z. electa* and *C. schineri*; however, these phylogenetic placements did not have bootstrap support. Analysis of mtDNA supported our previous hypothesis that *R. batava* and *R. flavigenualis* have phylogenetic affinity to five taxonomically-defined North American species groups. In addition, mtDNA data grouped *R. almatensis* and *R. flavicincta* with *R. cerasi* (L.), a result consistent with our present analysis of morphology. Analyses of both morphology and mtDNA place *R. magniterebra* as a sister taxon to *R. meigenii* (Loew). The Neotropical *Rhagoletis* species form a distinct group based on mtDNA analysis and, with the exception of the placement of *Rh. pastranai*, these relationships are consistent with morphology. Several clades observed in the morphological analysis were in conflict with those observed in the mtDNA analysis. Partition homogeneity tests indicated that significantly different phylogenetic signals emanate from the morphological and mtDNA data; therefore these data sets were not combined for analysis. Recent phylogenetic analyses of several *Rhagoletis* spp. based on DNA sequences of alleles at anonymous nuclear loci indicate that random genetic drift and/or hybridization and introgression may be playing a large role in the evolution of *Rhagoletis* species. We argue that these forces, combined with differential selection, can lead to fixation of alternate alleles in different lineages and result in the phylogenetic conflicts observed in the morphological and mtDNA analyses.

INTRODUCTION

The phylogenetic relationships of *Rhagoletis* spp. and related species in the tephritid subtribe Carpomyina are not well characterized. While a number of studies have examined morphological characteristics of members of the Carpomyina in a phylogenetic context (Berlocher, 1981; Jenkins, 1996; Norrbom, 1994, 1997), this work has not led to a broad consensus of phylogenetic relationships. The phylogenies proposed in these studies are also noteworthy for the lack of character support, especially for branches defining clades closer to the base of the tree. Mitochondrial DNA data give some support to some groups but intergroup relationships are not well supported (Smith and Bush, 1997); incomplete taxon sampling was presumably the cause of the weak inferences.

We previously proposed (Smith and Bush, 1999) hypotheses of relationship for 87 taxa in the Carpomyina based on a parsimony analysis of the morphological data set of Jenkins (1996). Our analysis was not unlike previous studies, leading to a tree that was poorly resolved due to a lack of strong character support. However, we were able to identify several hypothetical phylogenetic relationships that could be tested using an independent set of characters (Table 1). Some of these proposed relationships were not unexpected, such as the placement of the Palearctic species *R. almatensis* Rohdendorf, which infests *Lonicera* spp., with *R. cerasi* (Linnaeus). However, some placements were unexpected. For example, several *Rhagoletis* species with Palearctic distributions, *R. magniterebra* (Rohdendorf), *R. flavicincta* (Loew), *R. flavigenualis* Hering, and *R. batava* Hering were placed in a group with Nearctic *Rhagoletis* spp.

Our goal in this paper is to analyze further the phylogenetic relationships of Carpomyina, focussing on the relationships of the taxa that were analyzed by morphology alone in a previous paper (Smith and Bush, 1999) but for which both morphological and mitochondrial DNA data are now available. Mitochondrial DNA sequences are now available from individuals representing

Table 1
Key phylogenetic relationships in Carpomyina as inferred from this study compared with morphology based placements of Smith and Bush (1999)*

	Placement in Smith and Bush (1999)	Placement in present study (character support[#])
Palearctic Taxa		
R. almatensis	Placed with *R. cerasi*	Placed with *R. cerasi* (morphology & mtDNA)
R. magniterebra	Placed with *R. cingulata* and *R. suavis* groups	Not placed with Nearctic taxa; placed with *R. meigenii* (morphology & mtDNA)
R. flavicincta	Placed with *R. ribicola* and *R. tabellaria* groups + *R. fausta*	Not placed with Nearctic taxa; placed with *R. cerasi* group (morphology & mtDNA)
R. flavigenualis	Placed sister to *R. berberis* and the *R. pomonella* group	Placed with Nearctic *Rhagoletis* spp. (morphology & mtDNA)
R. batava	Placed with North American taxa	Placed with Nearctic *Rhagoletis* spp. (morphology & mtDNA)
Neotropical Taxa		
R. blanchardi	These five neotropical	These five neotropical
R. ferruginea	*Rhagoletis* apparently form a	*Rhagoletis* apparently form a
R. lycopersella	phylogenetically coherent group	phylogenetically coherent group
R. nova	(except *Rh. pastranai* anomaly)	(except *Rh. pastranai* anomaly)
R. psalida		Relationships of *R. rhytida* and *R. nova* groups unclear
Other Relationships		
R. alternata &	Placed as a sister group	Not placed as a sister group
R. basiola	to all *Rhagoletis*	to all *Rhagoletis*
genus *Rhagoletis*	Not monophyletic (*sensu* Hennig)	Not monophyletic (*sensu* Hennig)

* Phylogenetic placements in this study compared with the placements inferred in Smith and Bush (1999).
[#] Indicates whether support for the relationship derives from analysis of morphology, mtDNA or both.

several of the Palearctic *Rhagoletis* species with unexpected phylogenetic placements in the morphological analysis. We also test in this paper the phylogenetic relationships of several Neotropical *Rhagoletis* spp. for which mitochondrial DNA sequences have recently become available (*R. blanchardi* Aczél, *R. ferruginea* Hendel, *R. lycopersella* Smyth, *R. nova* (Schiner), and *R. psalida* Hendel). Analysis of morphology (Jenkins, 1996, Smith and Bush, 1999) indicated a close relationship of *Rh. pastranai* Aczél to these Neotropical *Rhagoletis* spp. In addition, we also test the hypothesis that the rose-infesting species *R. alternata* Fallén and *R. basiola* (Osten Sacken) form a sister group to the remainder of the genus *Rhagoletis*, a relationship recovered in the morphological analysis (Smith and Bush, 1999).

Finally, our previous work indicated that morphological and mitochondrial character evolution might be uncoupled in *Rhagoletis*. To examine this further, we conducted a partition homogeneity test to determine whether the mitochondrial DNA data and morphological data contained significantly different signals. The results of this test indicated that the mitochondrial DNA data and morphological data are not homogeneous. Thus, we could not justify analyzing the characters from these two data sources as a combined data set.

MATERIALS AND METHODS

Taxon sample

The morphological data set in Smith and Bush (1999) consisted of the 77 phylogenetically informative morphological characters collected by Jenkins (1996) from 87 taxa in the Carpomyina. In this paper the analysis is based on a subset of 43 of those 87 taxa for which mitochondrial DNA sequences in the COI/COII region are now available. The sample consists of 39 *Rhagoletis* spp. plus *C. schineri*, *O. latifrons*, *Rh. pastranai*, and *Z. electa* with *Epochra canadensis* (Loew) as the outgroup (Table 2).

Phylogenetic analysis of morphology

Analysis of morphology in the data set was based on the same set of characters described by Jenkins (1996). The morphological characters and their alternate states are described in Appendix 1. The aligned morphological data are included as Table 3 and are available via TreeBase (http://www.treebase.org/) as Study Accession # S914 and Matrix Accession # M1513.

Morphological characters were analyzed as discrete characters using parsimony as implemented in PAUP*4.0b3a (Swofford, 2000). All characters were coded as unordered (nonadditive) and parsimony was carried out on the phylogenetically informative character set

Table 2
Taxa included in this study (Abbreviations: PA-Palearctic, NA-Nearctic, NT-Neotropical)

Genus/Species	Host Plant Family	Distribution	GenBank Accession
Carpomya Costa			
C. schineri (Loew)	Rosaceae	PA: Central Europe to Kazakhstan & Israel	U53267
Epochra Loew			
E. canadensis (Loew)	Saxifragaceae	NA: Northeast-Northwest North America	U53265
Oedicarena Loew			
O. latifrons (Wulp)	Solanaceae	NA: Southwestern USA–Mexico	U53266
Rhagoletis Loew			
R. alternata Group			
R. alternata Fallén	Rosaceae	PA: Europe, Altai, Southern Siberia	U53260
R. basiola (Osten Sacken)	Rosaceae	NA: Western–Eastern North America	U53261
R. cerasi Group			
R. almatensis Rohdendorf	Caprifoliaceae	PA: Southeastern Kazakhstan-Northern Kirghizia	AY310718
R. berberidis Jermy	Berberidaceae	PA: Central-Eastern Europe	U53258
R. cerasi (Linnaeus)	Caprifoliaceae/ Rosaceae	PA: Europe	U53257

Table 2-cont.

Genus/Species	Host Plant Family	Distribution	GenBank Accession
R. cingulata Group			
R. cingulata (Loew)	Rosaceae	NA: Northeastern USA-North Central Mexico	U53248
R. chionanthi Bush	Oleaceae	NA: Southeastern USA	U53251
R. indifferens Curran	Rosaceae	NA: Northwestern USA-Southwestern Canada	U53249
R. osmanthi Bush	Oleaceae	NA: Southeastern USA	U53250
R. ferruginea Group			
R. blanchardi Aczél	Solanaceae	NT: Argentina	AY310720
R. ferruginea Hendel	Solanaceae	NT: Brazil	AY310721
R. flavicincta Group			
R. flavicincta (Loew)	Caprifoliaceae	PA: Europe, Russia, Kazakhstan	AY310722
R. meigenii Group			
R. meigenii (Loew)	Berberidaceae	PA: Europe, Caucasus	U53259
R. nova Group			
R. conversa (Brèthes)	Solanaceae	NT: Central Chile	U53263
R. lycopersella Smyth	Solanaceae	NT: Peru	AY310724
R. nova (Schiner)	Solanaceae	NT: Central Chile	AY310726
R. pomonella Group			
R. cornivora Bush	Cornaceae	NA: USA-Southeastern Canada	U53238
R. mendax Curran	Ericaceae	NA: USA-Southeastern Canada	AY310728
R. nr. *mendax*	Cornaceae	NA: Eastern USA	U53237
R. pomonella (Walsh)	Rosaceae	NA: Eastern USA-Southeastern Canada	U53229
R. zephyria Snow	Caprifoliaceae	NA: North Central-Northeastern-USA-Southeastern Canada	U53234
R. psalida Group			
R. psalida Hendel	Solanaceae	NT: Peru-Bolivia	AY310727
R. ribicola Group			
R. berberis Curran	Berberidaceae	NA: Northwestern USA-Southwestern Canada	U53247
R. ribicola Doane	Saxifragaceae	NA: Northwestern USA-Southwestern Canada	U53246
R. striatella Group			
R. striatella Wulp	Solanaceae	NA: Central-Eastern USA-Northern Mexico	U53262
R. suavis Group			
R. boycei Cresson	Juglandaceae	NA: Southwestern USA-North Central Mexico	U53254
R. completa Cresson	Juglandaceae	NA: South Central - North Central USA-Northeastern Mexico	U53256
R. juglandis Cresson	Juglandaceae	NA: Southwestern USA-	U53253

Table 2 (cont.)

Genus/Species	Host Plant Family	Distribution	GenBank Accession
		North Central Mexico	
R. suavis (Loew)	Juglandaceae	NA: Eastern USA	U53252
R. zoqui Bush	Juglandaceae	NT: East Central Mexico	U53255
R. tabellaria Group			
R. electromorpha Berlocher	Cornaceae	NA: North Central USA	U53242
R. persimilis Bush	Liliaceae	NA: Northwestern USA-Southwestern Canada	U53244
R. tabellaria (Fitch)	Cornaceae	NA: Northwestern USA-Southwestern Canada	U53239
R. nr. *tabellaria*	Eleagnaceae	NA: Northwestern USA-Southwestern Canada	U53245
R. zernyi Group			
R. flavigenualis Hering	Cupressaceae	PA: Turkey, Northwestern Caucasus	AY310723
Unplaced *Rhagoletis*			
R. batava Hering	Rhamnaceae	PA: North Central-Eastern Europe, Northern Caucasus, Kirghizia	AY310719
R. fausta (Osten Sacken)	Rosaceae	NA: Northeastern USA-Northwestern USA	U53264
R. juniperina Marcovitch	Cupressaceae	NA: North Central-Northeastern USA-South Central-Southeastern USA	U53243
R. magniterebra (Rohdendorf)	Berberidaceae	PA: Kazakhstan, Kirghzia, Northern Tadjikistan	AY310725
Rhagoletotrypeta Aczél			
Rh. pastranai Aczél	Ulmaceae	NT: Southern Brazil-Northern Argentina	U53268
Zonosemata Benjamin			
Z. electa (Say)	Solanaceae	NA: Eastern USA, Southeastern Canada	U53265

using the ACCTRAN character state optimization option. Constant characters are a subset of phylogenetically uninformative characters. When all character states are the same among taxa, the character is considered to be "constant". When only a single taxon has a different character state, the character is also phylogenetically uninformative, because it can provide no information about group membership. Neither set provides information used to identify clades in a parsimony analysis, but uninformative characters can give information about the amount of evolution that has occurred along a particular branch in a tree (while constant characters do not).

Trees were obtained using the heuristic search option with random sequence addition and TBR branch swapping. To discover if multiple islands of most parsimonious trees existed, 10

replicates of the random addition procedure were performed. Initial searches were performed with each character weighted equally. The data were analyzed subsequently using the successive approximations method of Farris (1969) to choose among equally parsimonious cladograms (Carpenter, 1988) obtained in the equal-weighting analysis. For this analysis, characters were weighted *a posteriori* on the basis of the maximum values of their rescaled consistency indices (Farris, 1989) within the set of most parsimonious reconstructions (MPRs) in the equal weighting analysis. Tree scores (treelength, consistency index and retention index) were calculated for each minimum length tree. Trees obtained in parsimony analysis of morphological features were summarized as strict consensus trees.

Phylogenetic analysis of mitochondrial DNA sequences

Mitochondrial DNA sequences in the COI/$tRNA_{Leu}$/COII region were analyzed from single individuals representing each of the 44 taxa in Table 2. Whenever possible, the DNA sequences analyzed were the same ones used in Smith and Bush (1997). Thirty-three of the sequences analyzed were expansions (additional nucleotides for COI and $tRNA_{Leu}$) of the COII sequences analyzed in Smith and Bush (1997). The GenBank records for these sequences were updated, and the nucleotide sequences for 11 new DNA sequences analyzed in this paper were deposited in GenBank. Accession numbers for all 44 sequences are shown in Table 2. The aligned mitochondrial DNA data set has been deposited with and is available from TreeBase (http://www.treebase.org/) as Study Accession # S914 and Matrix Accession # M1512. DNA sequences used in the analysis were obtained from individual flies using methods described in Smith and Bush (1997). DNA amplifications using the polymerase chain reaction were accomplished using the George-Eva primer pair.

Mitochondrial DNA data were analyzed as discrete characters by maximum parsimony as described above using PAUP*4.0b3a (Swofford, 2000). Again, characters were coded as unordered (nonadditive) and parsimony was carried using the ACCTRAN character state optimization option. Separate analyses were carried out with gaps positions treated as an extra character state (5^{th} nucleotide) or as missing data. Branch support for clades in the MPRs was determined by performing 100 bootstrap replicates (Felsenstein, 1985).

Mitochondrial DNA data were also analyzed by neighbor-joining analysis of pairwise genetic distances using PAUP*4.0b3a (Swofford, 2000). For these analyses, Kimura's 2-parameter distances were used, which correct for multiple substitutions and take into consideration differences in rates of transitional and tranversional nucleotide substitutions. Both pairwise deletion of gaps and complete deletion of gaps were used in the distance calculations. Branch support was assessed by bootstrapping in PAUP*4.0b3a with 1000 pseudoreplicate data sets.

Partition homogeneity test of combinability

The partition homogeneity test as implemented in PAUP*4.0b3a (Swofford, 2000) was used to determine whether or not the morphological data and the mtDNA data could justifiably be combined for subsequent analysis. This is the incongruence length difference test of Farris *et al.* (1995), which involves assessing independently the tree lengths for the morphology "partition" and the mtDNA "partition". A number of random partitions are then created (99) in which the morphological and mtDNA characters are randomly assigned to one partition or the other. The sums of the tree lengths from these random partitions are then compared with the sum obtained in the original data set.

Table 3
Morphological character matrix used in the parsimony analysis

Character Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Taxon																							
R.almatensis	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	01	1	0	0	1	1
R.alternata	01	0	0	01	1	01	01	0	0	0	0	0	0	0	0	0	0	01	01	0	0	1	0
R.basiola	1	0	0	1	1	01	01	0	0	0	0	0	0	0	0	0	0	01	1	0	0	1	0
R.batava	1	0	0	01	1	1	01	0	0	0	1	0	0	0	0	0	0	01	1	0	1	1	01
R.berberidis	1	0	0	0	01	0	0	0	0	0	1	0	0	0	0	0	0	01	01	0	0	1	1
R.berberis	1	0	0	01	1	01	0	0	0	0	1	0	0	0	0	0	0	01	01	0	1	1	1
R.blanchardi	1	1	0	0	1	0	0	0	0	0	01	0	0	0	0	0	0	0	1	0	0	1	0
R.boycei	01	0	0	0	1	1	01	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1
R.cerasi	1	0	0	0	01	0	0	0	0	0	1	0	0	0	0	0	0	0	01	0	0	1	1
R.chionanthi	1	0	0	1	1	1	01	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1
R.cingulata	1	0	0	1	1	1	01	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1
R.completa	1	0	0	01	1	1	1	01	0	0	01	0	0	0	0	0	0	1	1	0	1	1	1
R.conversa	1	1	0	0	1	01	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	01
R.cornivora	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	1	1	1	0	1	1	1
R.electromorpha	1	0	0	01	1	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	1
R.fausta	01	0	0	01	1	1	0	0	0	0	1	0	0	0	0	0	0	01	1	0	1	1	1
R.ferruginea	1	1	0	01	1	01	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
R.flavicincta	1	0	0	01	1	1	1	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1
R.flavigenualis	1	0	0	1	1	1	01	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1
R.indifferens	1	0	0	1	1	1	01	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1
R.juglandis	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1
R.juniperina	1	0	0	01	1	1	0	0	0	0	1	0	0	0	0	0	0	01	1	0	1	1	1
R.lycopersella	01	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1
R.magniterebra	1	0	0	1	1	1	01	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	1
R.meigenii	01	0	0	1	1	1	1	01	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1
R.mendax	1	0	0	1	1	1	01	0	0	0	1	0	0	1	0	0	1	1	1	0	1	1	1
R.nova	1	1	0	1	1	1	01	0	0	0	1	0	0	0	0	0	0	01	1	0	0	1	0
R.nr.mendax	01	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	1	1	1	0	1	1	1
R.nr.tabellaria	1	0	0	1	1	1	01	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1
R.osmanthi	1	0	0	1	1	1	0	01	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1
R.persimilis	1	0	0	01	1	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	1
R.pomonella	01	0	0	1	1	1	0	0	0	0	1	0	0	01	0	0	1	1	1	0	1	1	1
R.psalida	01	1	0	1	1	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	1	1
R.ribicola	01	0	0	1	1	1	01	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1
R.striatella	1	0	0	01	1	01	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	1	1
R.suavis	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	01
R.tabellaria	01	0	0	01	1	1	0	0	0	0	1	0	0	0	0	0	0	01	1	0	1	1	1
R.zephyria	1	0	0	01	1	1	0	0	0	0	1	0	0	1	0	0	1	1	1	0	1	1	1
R.zoqui	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0
C.schineri	1	0	0	1	1	1	1	0	0	0	0	1	0	0	1	1	0	1	1	0	1	1	1
E.canadensis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Rh.pastranai	0	1	0	0	01	0	0	0	0	0	1	2	0	1	0	0	0	0	0	0	0	1	1
O.latifrons	0	0	0	0	0	0	0	0	0	0	01	0	0	0	0	0	0	0	0	1	0	1	0
Z.electa	1	0	0	01	1	1	0	0	0	0	0	2	1	1	0	0	0	01	1	0	0	1	0

24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	01	0	01	0	0	1	1	0	1	0	0	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	1
0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	1	0	0	1
0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1
0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	01	1	0	01	1	0	0	1
0	0	0	0	01	0	0	0	1	0	1	1	?	0	0	0	0	1	0	0	1	0	?	0	0	0	1
0	0	0	1	01	0	0	0	0	0	1	1	0	0	0	0	1	1	1	0	1	1	0	1	1	0	1
0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	01	1	0	0	1
0	0	1	0	01	0	0	0	0	0	1	1	01	0	0	0	0	1	1	01	1	1	0	1	1	0	1
0	0	1	01	01	0	0	0	0	0	01	1	0	0	0	0	0	1	1	01	1	1	0	1	1	0	1
0	0	0	01	1	0	0	0	0	0	01	1	0	0	0	0	1	1	1	0	1	1	0	1	1	0	1
0	0	0	1	01	0	0	0	0	0	1	1	0	0	0	0	0	1	0	01	1	0	0	1	0	0	1
0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	01	1	0	0	1	0	0	1
0	0	0	1	0	1	0	0	0	0	1	1	0	1	0	0	0	1	1	1	1	1	1	1	0	0	1
0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	1
0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1
0	0	0	1	0	0	0	0	0	0	01	01	0	0	0	0	0	1	0	1	1	0	1	1	0	0	1
0	0	0	01	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	0	0	1	0	0	1
0	0	1	1	0	0	0	0	0	0	1	1	01	0	0	0	0	1	1	01	1	1	0	1	1	0	1
0	0	0	0	01	0	0	0	0	0	0	1	0	0	0	0	1	1	1	01	1	1	0	1	1	0	1
0	0	0	1	01	0	0	0	0	0	1	01	0	0	0	0	0	1	1	01	1	0	0	1	0	0	1
0	0	0	1	01	0	0	0	0	1	1	1	0	0	0	0	0	1	0	1	1	0	0	1	0	0	1
0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	0	1	0	0	1
0	0	0	0	01	1	0	0	0	0	0	0	0	0	0	0	0	1	1	01	1	1	0	1	0	0	1
0	1	0	1	01	0	0	0	0	0	1	1	0	0	0	0	0	1	0	01	1	1	0	1	0	0	1
0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1
0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	01	1	0	0	1	0	0	1
0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	1	0	1	0	0	1
0	0	1	01	01	0	0	0	0	0	01	1	0	0	0	0	0	1	1	0	1	1	0	1	1	0	1
0	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	1	0	1	0	0	1
0	1	0	1	01	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	1	1	0	1	0	0	1
0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1
0	0	0	01	0	1	0	0	0	0	1	1	0	1	1	0	0	1	1	1	1	0	0	1	0	0	1
0	0	0	1	1	0	0	0	0	0	1	1	0	0	01	0	0	1	0	01	1	0	0	1	0	0	1
0	0	0	1	0	0	0	0	0	0	01	0	0	0	0	0	1	1	1	1	1	01	0	1	1	0	1
0	0	0	1	0	1	0	0	0	0	1	1	01	1	0	0	0	1	1	01	1	1	1	1	0	0	1
0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	01	1	01	0	1	0	0	1
0	0	0	01	1	0	0	0	0	0	0	1	0	0	0	0	1	1	1	1	1	1	0	1	1	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	01	0	0	1	0	0	1	0	0	1	0	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	1	1	0	1	0	0	1
0	0	0	1	0	1	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	01	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1

Table 3 (cont.)

Character Number	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77
Taxon																											
R.almatensis	1	0	1	0	0	0	0	0	1	0	0	4	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0
R.alternata	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1	1	0	1	0	0	0	0	1	0	0	0	0
R.basiola	1	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0
R.batava	1	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	01	0	0	0	0	1	0	0	0	0
R.berberidis	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1	1	0	0	1
R.berberis	1	0	1	0	0	0	0	0	1	0	0	2	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0
R.blanchardi	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
R.boycei	1	0	1	0	0	0	0	0	1	0	0	3	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0
R.cerasi	1	0	1	0	0	0	0	0	1	0	0	4	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0
R.chionanthi	1	0	1	0	0	0	0	0	1	0	0	3	1	0	0	1	0	01	0	0	0	0	1	0	0	0	0
R.cingulata	1	0	1	0	0	0	0	0	1	0	0	3	1	0	0	1	0	01	0	0	0	0	1	0	0	0	0
R.completa	1	0	1	0	0	0	0	0	1	0	0	3	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0
R.conversa	1	0	1	0	0	0	0	0	1	0	0	2	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0
R.cornivora	1	0	1	0	0	0	0	0	1	0	0	2	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0
R.electromorpha	1	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0
R.fausta	1	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0
R.ferruginea	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1	1	0	1	0	0	0	0	01	0	0	0	0
R.flavicincta	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1	1	0	0	0
R.flavigenualis	1	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	1
R.indifferens	1	0	1	0	0	0	0	0	1	0	0	3	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0
R.juglandis	1	0	1	0	0	0	0	0	1	0	0	2	0	0	0	1	0	01	0	0	0	0	1	01	0	0	0
R.juniperina	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1	1	0	01	0	0	0	0	1	0	0	0	0
R.lycopersella	1	0	1	0	0	0	0	0	1	0	0	2	1	0	1	1	0	1	0	0	0	0	1	0	0	0	0
R.magniterebra	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
R.meigenii	1	0	1	0	0	0	2	0	1	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	0	0	1
R.mendax	1	0	1	0	0	0	0	0	1	0	0	2	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0
R.nova	1	0	1	0	0	0	0	0	1	0	0	2	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0
R.nr.mendax	1	0	1	0	0	0	0	0	1	0	0	2	0	0	0	1	0	1	0	0	0	0	1	01	0	0	0
R.nr.tabellaria	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	01	0	0	0
R.osmanthi	1	0	1	0	0	0	0	0	1	0	0	3	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0
R.persimilis	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	01	0	0	0	0	1	1	0	0	0
R.pomonella	1	0	1	0	0	0	0	0	1	0	0	2	0	0	0	1	0	1	0	0	0	0	1	01	0	0	0
R.psalida	1	0	1	0	0	0	0	0	1	0	0	2	1	0	1	1	0	1	0	0	0	0	1	0	0	0	0
R.ribicola	1	0	1	0	0	0	0	0	1	0	0	2	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0
R.striatella	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0
R.suavis	1	0	1	0	0	0	0	0	1	0	0	2	0	0	0	1	0	1	0	0	0	0	1	01	0	0	0
R.tabellaria	1	0	1	0	0	0	0	0	1	0	0	0	1	01	0	1	0	0	0	0	0	0	1	0	0	0	0
R.zephyria	1	0	1	0	0	0	0	0	1	0	0	2	0	0	0	1	0	1	0	0	0	0	1	01	0	0	0
R.zoqui	1	0	1	0	0	0	0	0	1	0	0	3	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0
C.schineri	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	0	1
E.canadensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rh.pastranai	1	0	1	0	0	0	0	0	1	0	0	2	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0
O.latifrons	1	0	0	0	1	0	1	1	0	0	0	0	2	1	0	1	0	1	0	0	0	0	0	0	0	0	0
Z.electa	0	0	0	0	0	0	0	0	1	0	1	2	1	0	1	1	1	1	0	0	0	0	0	0	1	0	0

RESULTS

Analysis of morphology

The morphological data set consisted of the 77 characters analyzed by Jenkins (1996), of which 47 were phylogenetically informative within the 44 taxon sample. Heuristic search of the tree space yielded 28,671 trees of length 283 (CI = 0.636, excluding uninformative characters; RI = 0.674). These trees resulted from three distinct islands (27,111, 1440, and 120 trees respectively). The trees in the first (27,111 trees) and second (1440 trees) islands were more similar to each other than either set was to the third island (120 trees) as judged by examination of consensus trees and comparisons of tree files using MacClade, vers. 3.08 (Maddison and Maddison, 1992).

Reweighting of characters based upon the maximum values of their rescaled consistency indices in the 28,671 trees yielded 11 characters with a weight of 1 and 36 characters with a weight less than 1. Heuristic search using the reweighted characters yielded 35,851 trees of length 30.51 (CI = 0.659, excluding uninformative characters; RI = 0.865). A strict consensus of these trees is shown in Fig. 1. The consensus tree obtained using weighted parsimony has 18 more defined clades than the consensus tree obtained using unweighted parsimony.

Analysis of mtDNA

The mtDNA data set consists of 1027 aligned nucleotide positions in the COI/COII region. Positions 1-180 correspond to the 3' end of COI, positions 188-253 correspond to $tRNA_{Leu}$, and positions 341-1027 correspond to COII. The intergenic region between COI and $tRNA_{Leu}$ was constant within the set of 44 taxa. However, the intergenic region between $tRNA_{Leu}$ and COII was highly variable within the data set. All positions with gaps are in the second intergenic region.

The mtDNA data set with gaps treated as missing data contained 292 phylogenetically informative characters. Parsimony analysis resulted in 21 trees of length 1181 (CI = 0.376, excluding uninformative characters; RI = 0.547). A strict consensus of these 21 trees contained 28 clades, with six of these being identical to clades recovered in the morphological analyses.

The mtDNA data were subsequently analyzed with gaps treated as a 5^{th} nucleotide, which resulted in a data set with 326 phylogenetically informative characters. Parsimony analysis resulted in 9 trees of length 1265 (CI = 0.383, excluding uninformative characters; RI = 0.575). A strict consensus of these 9 trees is shown in Fig. 2. In this analysis, 6 clades were defined in addition to those identified in the "gaps missing" analysis.

The mtDNA data were also analyzed using neighbor joining. The neighbor-joining tree obtained with distances calculated using pairwise-deletion of gapped positions is shown as a rooted phylogram in Fig. 3. When gap positions were eliminated from the analysis, the neighbor-joining tree obtained was nearly identical to the tree shown in Fig. 3. Only the positions of *R. fausta* (becomes sister to *R. tabellaria* species group), *R. ribicola* (clusters with *R. berberis*) and *Rh. pastranai* (becomes sister to *R. berberidis*/*R. striatella*/*O. latifrons* and South American *Rhagoletis*) change in the analysis. These are relatively minor changes and, in each case, there is no bootstrap support for preferring one resolution (pairwise-deletion) of these taxa to the other (complete-deletion).

The neighbor-joining tree is also shown in Fig. 4 as an unrooted phylogram. This representation provides a different perspective on the inferred relationships of the carpomyine species, and shows very well the polytomous nature of the tree.

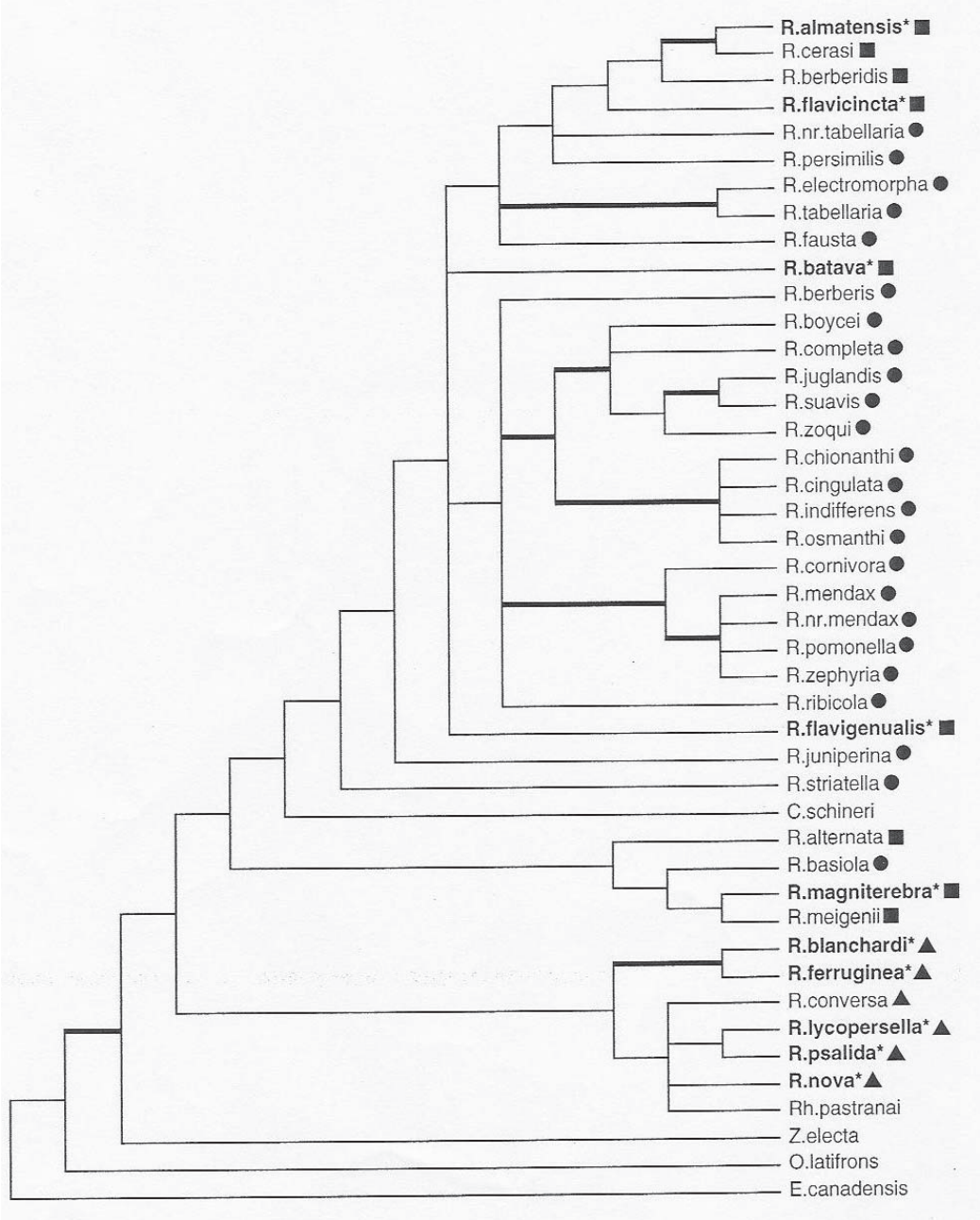

Fig. 1. Parsimony analysis of Carpomyina based on morphological characters. Tree shown is a strict consensus of 35,873 trees obtained via successive approximations. Branches supported in a parsimony analysis of unweighted characters are shown in bold. Starred (*) taxa in bold indicate those taxa that have not been previously analyzed using mitochondrial DNA characters. Predominantly Nearctic *Rhagoletis* taxa are indicated with ●, predominantly Palearctic *Rhagoletis* taxa are indicated with ■, and predominantly Neotropical *Rhagoletis* taxa are indicated with ▲.

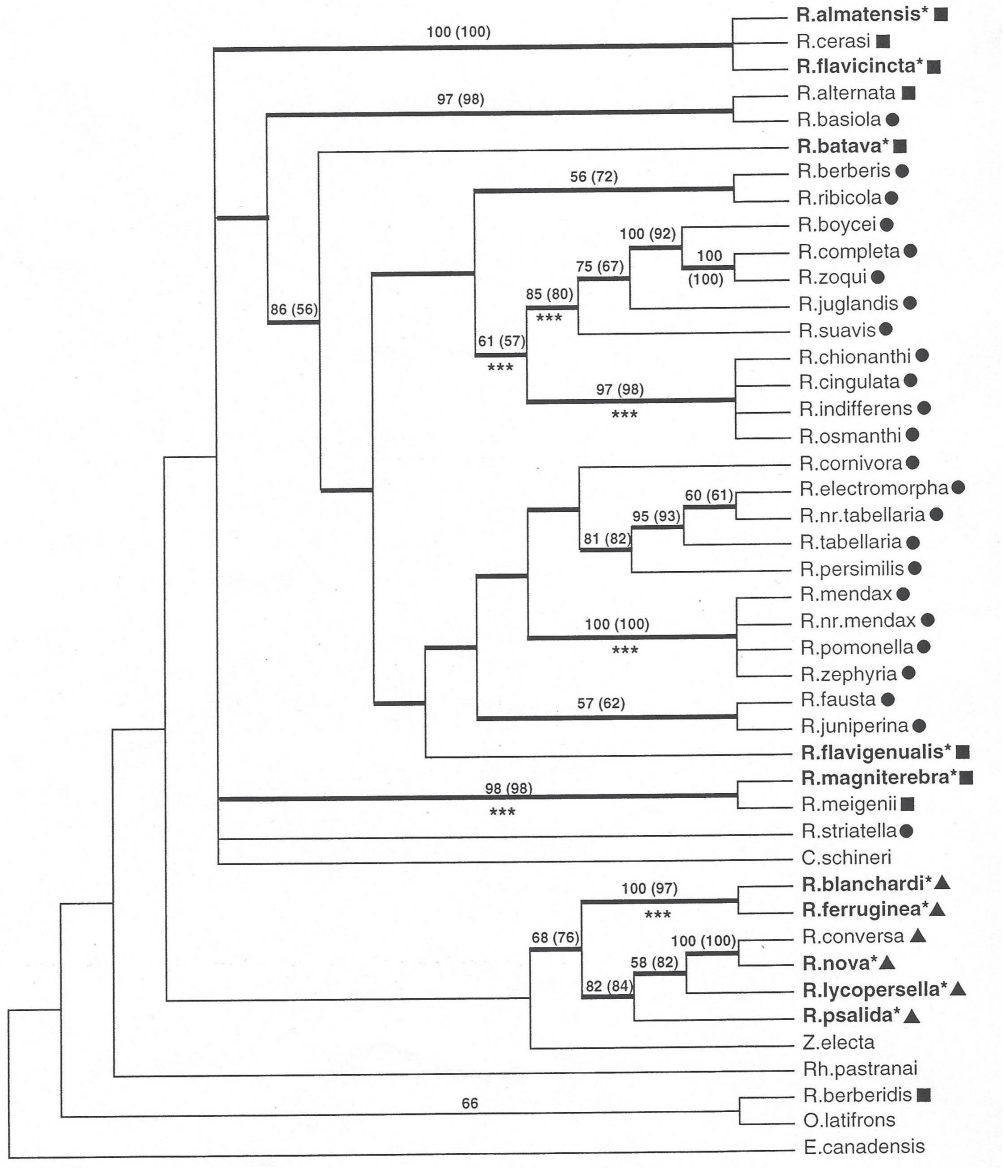

Fig. 2. Parsimony analysis of Carpomyina based on mitochondrial DNA characters. Tree shown is strict consensus of 9 trees obtained treating gap positions in the aligned DNA sequences as a 5th nucleotide. Branches shown in bold are those that are supported in a parsimony analysis of the mtDNA data set with gap positions treated as missing data. Numbers on branches are parsimony bootstrap values, with numbers in parentheses representing bootstrap values obtained in the analysis with gaps treated as missing data. Branches indicated with three stars (***) are those branches that are also present in the tree obtained via parsimony analysis of morphological characters. Starred (*) taxa in bold indicate those taxa that have not been previously analyzed using mitochondrial DNA characters. Predominantly Nearctic *Rhagoletis* taxa are indicated with ●, predominantly Palearctic *Rhagoletis* taxa are indicated with ■, and predominantly Neotropical *Rhagoletis* taxa are indicated with ▲.

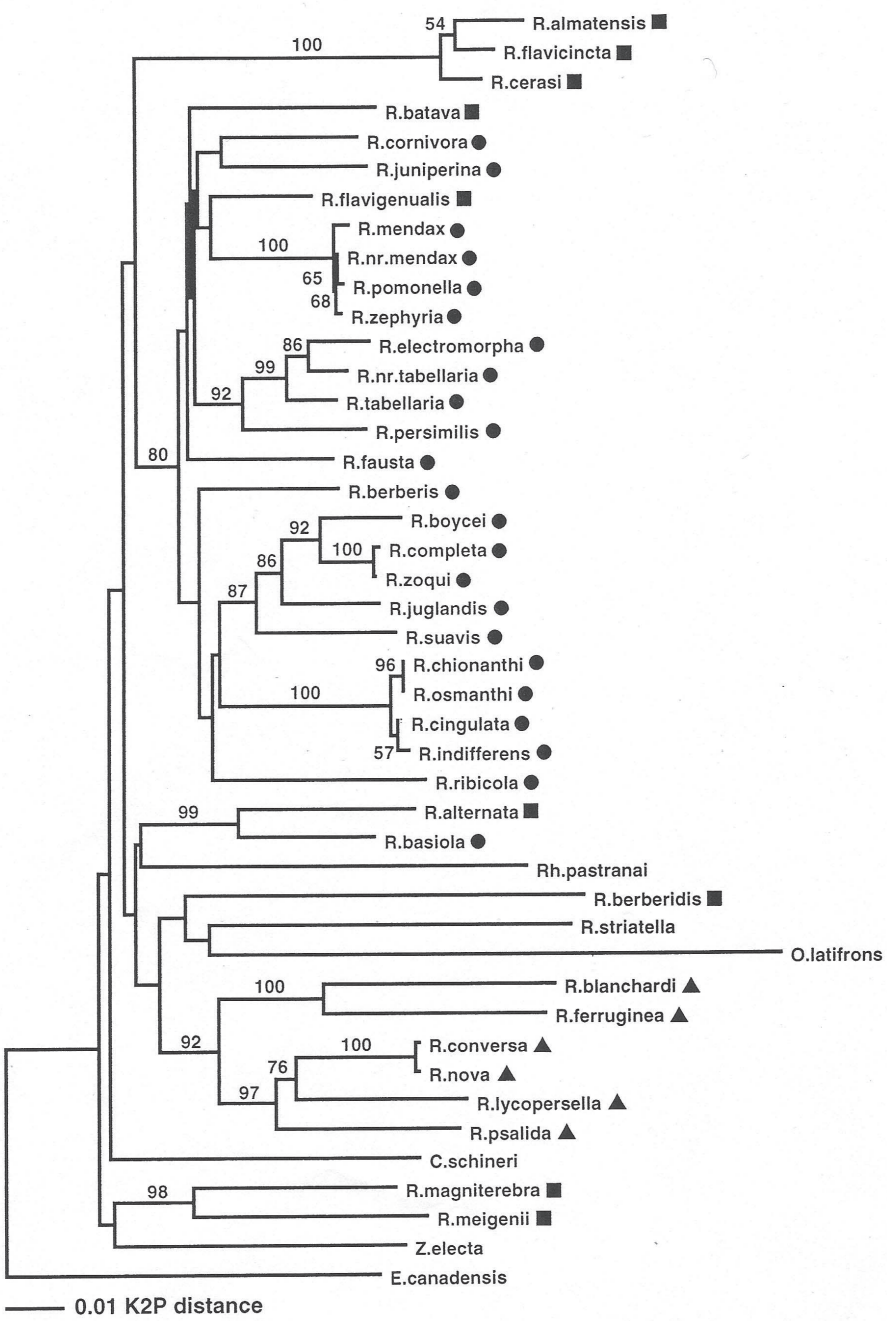

Fig. 3. Neighbor-joining analysis of Carpomyina based on mtDNA data set. Tree shown as a rooted phylogram with branch lengths proportional to Kimura 2-parameter distance (scale bar). Neighbor-joining bootstrap values >50 (1000 replicates) are indicated on branches. Predominantly Nearctic *Rhagoletis* taxa are indicated with ●, predominantly Palearctic *Rhagoletis* taxa are indicated with ■, and predominantly Neotropical *Rhagoletis* taxa are indicated with ▲.

Partition homogeneity test

A partition homogeneity test was employed to assess whether or not the morphological and mtDNA characters contain conflicting phylogenetic signals. The results of 100 partitions are shown in Fig. 5. When the morphological data and the mtDNA data were analyzed, the sum of the tree lengths was 1548 (283 steps for unweighted parsimony of morphology, 1265 steps for parsimony of mtDNA with gaps treated as a 5th nucleotide). The 99 random partitions all had combined treelengths that were longer than 1548 (range 1553-1574). The *p*-value associated with this result is 0.01.

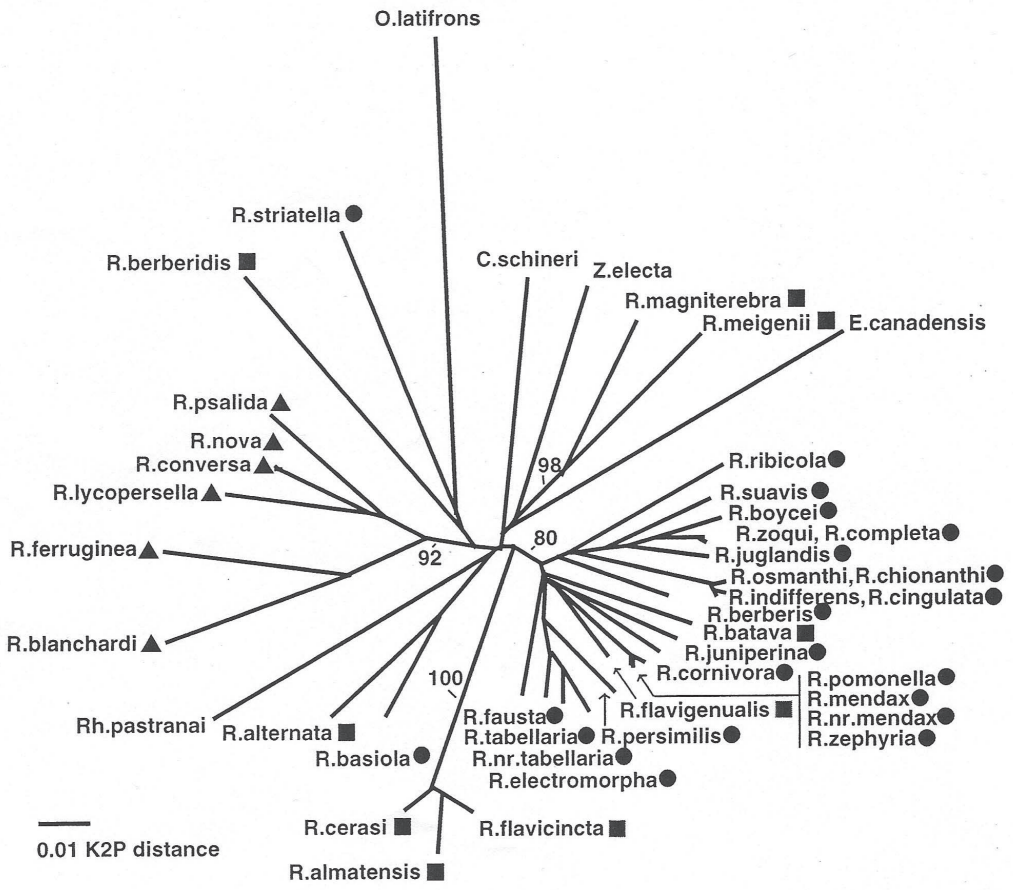

Fig. 4. Neighbor-joining analysis of Carpomyina based on mtDNA data set. Tree shown as an unrooted phylogram with branch lengths proportional to Kimura 2-parameter distance (scale bar). Neighbor-joining bootstrap values >50 (1000 replicates) for major clusters are indicated on branches. Predominantly Nearctic *Rhagoletis* taxa are indicated with ●, predominantly Palearctic *Rhagoletis* taxa are indicated with ■, and predominantly Neotropical *Rhagoletis* taxa are indicated with ▲.

DISCUSSION

Previous studies (Jenkins, 1996, Smith and Bush, 1997, 1999) examined morphological and mitochondrial DNA relationships of *Rhagoletis* spp. and their relatives in the tephritid subtribe Carpomyina. This study extends the previous work to incorporate mtDNA data that were not available before from several Palearctic *Rhagoletis* species (*R. almatensis*, *R. flavigenualis*, *R. flavicincta*, *R. batava* and *R. magniterebra*) and Neotropical *Rhagoletis* species (*R. nova*, *R. psalida*, *R. lycopersella*, *R. blanchardi* and *R. ferruginea*). The data are analyzed in a hypothesis-testing framework to test explicitly several proposed phylogenetic relationships arising in the previous work (Table 1).

Palearctic *Rhagoletis*

Morphological and mtDNA characters (Figs. 1, 2) both support the phylogenetic affinity of the Palearctic *Rhagoletis* species, *R. batava* and *R. flavigenualis*, to the Nearctic *Rhagoletis* (excluding *R. basiola* and *R. striatella*). Analyzing pairwise distances via neighbor-joining (Figs. 3 and 4) illustrates this phylogenetic affinity and allows visualization of the relative divergence times of the different lineages.

Inclusion of *R. batava* and *R. flavigenualis* in the data set disrupted the monophyly (*sensu* Hennig), proposed in earlier work (Smith and Bush, 1997, McPheron and Han, 1997), of five taxonomically-defined species groups (*pomonella, tabellaria, cingulata, suavis,* and *ribicola*) plus *R. juniperina* and *R. fausta* (here referred to as the *R. pomonella* supergroup). While all

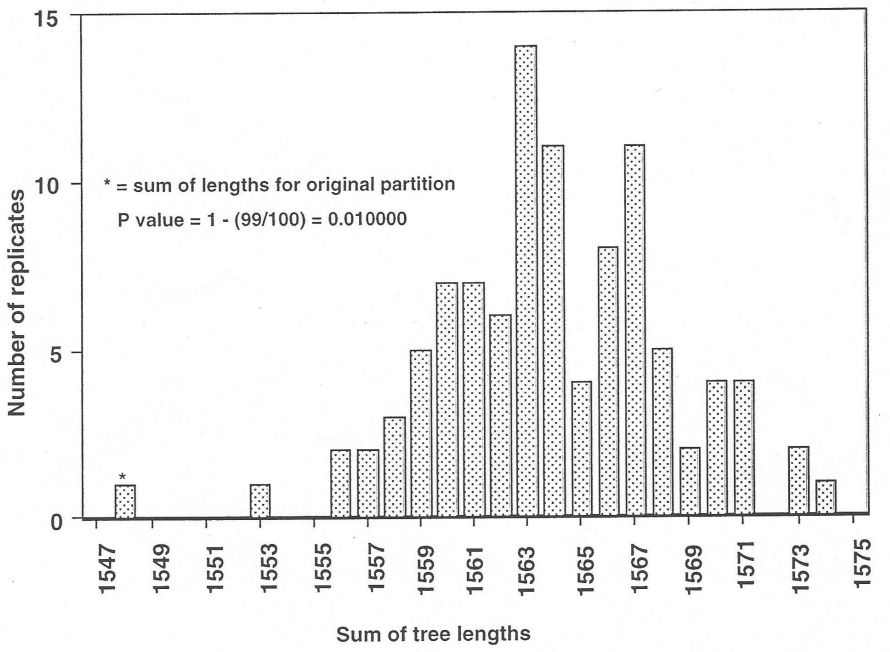

Fig. 5. Incongruence of the morphological and mitochondrial DNA data sets. The x-axis indicates the sum of the tree lengths obtained from the original data partition (*) and the 99 random partitions. The y-axis indicates the number of times each summed tree length was observed.

members of the *R. pomonella* supergroup are exclusively Nearctic in distribution, it appears that these Nearctic *Rhagoletis* are not monophyletic (*sensu* Hennig), and that *Rhagoletis* species were in fact undersampled in the earlier studies, as suggested in Smith and Bush (1999). The host relationships of *R. batava* and *R. flavigenualis* may shed light on ancestral host use in the *R. pomonella* supergroup. *R. batava* infests *Hippophae rhamnoides* (Rhamnaceae) while *R. flavigenualis* infests *Juniperus* spp. (Cupressaceae). Plants in both of these families apparently are infested by species in the *R. pomonella* supergroup. *R. juniperina* infests *Juniperus* spp. in eastern North America, and one of us (GB) has recently observed larvae of an undescribed *Rhagoletis* sp. in fruits of buckthorn (*Rhamnus* sp.) in Washington State.

Analysis of morphology and mtDNA data supports grouping *R. almatensis* and *R. flavicincta* with *R. cerasi* (Figs. 1, 2). These data support previously proposed hypotheses with respect to the relationship of the *Lonicera*-infesting *R. almatensis*, but the present placement of *R. flavicincta* contradicts our earlier hypothesis (Smith and Bush, 1999), in which *R. flavicincta* was placed with the Nearctic *Rhagoletis*. The placement of *R. flavicincta* in the present study is strongly supported by the mtDNA data, with bootstrap values of 100 in both the parsimony and neighbor-joining analyses (Figs. 2 and 3). It may be that the *Lonicera*-infesting *Rhagoletis* in Eurasia form a phylogenetically cohesive group.

The present analyses also conflict with our previous hypothesis (Smith and Bush, 1999) with respect to the placement of *R. magniterebra* as a sister taxon to the Nearctic *R. cingulata* and *R. suavis* species groups. Analyses of both morphology and mtDNA (Figs. 1, 2) place *R. magniterebra* as a sister taxon to *R. meigenii*. The mtDNA bootstrap values for this grouping were 98 and 97, respectively, in the parsimony and the neighbor-joining analyses (Figs. 2, 3). Again, we see phylogenetic affinity between species that infest similar hosts; *R. meigenii* infests *Berberis vulgaris* while *R. magniterebra* infests *B. heteropoda*.

Neotropical *Rhagoletis*

One of the major anomalies of our morphological systematics studies of Neotropical Carpomyina has been the placement of *Rhagoletotrypeta pastranai*, which was placed with *R. nova* group species when the complete data set of Jenkins (1996) was analyzed (Smith and Bush, 1999). The same result was obtained here with the abbreviated set of 43 ingroup taxa (Fig. 1). However, analysis of mtDNA provides no support for grouping *Rh. pastranai* with the *R. nova* group species (Figs. 2 and 3). In the parsimony analysis of mtDNA, *Rh. pastranai* is placed as a sister taxon to a clade consisting of all of the *Rhagoletis* species (except *R. berberidis*), *Carpomya schineri* and *Zonosemata electa*. Forcing *Rh. pastranai* into a clade with *R. conversa*, *R. nova*, *R. psalida* and *R. lycopersella*, as in the morphology tree (Fig. 1), results in a tree of length 1193, which is 12 steps longer than the most parsimonious trees of 1181 steps (gaps treated as missing).

Aside from the *Rh. pastranai* anomaly, the South American *Rhagoletis* species appear to form a coherent monophyletic group. However, placements of species within this monophyletic group vary depending on the source of the characters used in the analysis. Analysis of morphology places *R. psalida* as a sister to *R. lycopersella*, with *R. nova* and *R. conversa* as sisters to this group (Fig. 1). On the other hand, the mtDNA data support a clade consisting of the three *R. nova* group species (*R. nova*, *R. conversa* and *R. lycopersella*), with *R. psalida* as the sister to this group. Interestingly, analysis of DNA sequences of alleles at an anonymous nuclear locus indicated that *R. psalida* is a sister to *R. conversa* and *R. nova*, with *R. lycopersella* the sister taxon to this clade (Jaycox *et al.*, personal communication). Clearly,

there are many unanswered questions with respect to the relationships of the South American *Rhagoletis* taxa.

Other relationships

Two other relationships that have been proposed previously have been re-examined in this study. The first is that the rose-infesting *R. alternata* and *R. basiola* form a sister group to all *Rhagoletis*. The second is that the genus *Rhagoletis* is not a monophyletic group (*sensu* Hennig).

Analysis of morphology places *R. alternata* and *R. basiola* in a clade with the *Berberis*-infesting *R. meigenii* and *R. magniterebra* (Fig. 1). The mtDNA data place *R. alternata* and *R. basiola* as a sister group to the Nearctic *Rhagoletis* (including *R. batava* and *R. flavigenualis*, excluding *R. striatella*; Fig. 2). However, there was no bootstrap support for this latter placement. In either case, there is no support from our present analyses that *R. alternata* and *R. basiola* form a sister group to all *Rhagoletis*.

Several past studies have presented phylogenetic hypotheses that indicate that the genus *Rhagoletis* is not monophyletic, in the strict holophyletic sense (*sensu* Hennig) commonly used in systematics studies (Berlocher and Bush, 1982, Jenkins, 1996, Han and McPheron, 1997, Smith and Bush, 1997). The analysis of the present data set also indicates that *Rhagoletis* may not be monophyletic. For example, in both the morphology tree (Fig. 1) and the mtDNA tree (Fig. 2) *Carpomya schineri* is placed deep within *Rhagoletis* (Fig. 1). Similarly, *Zonosemata electa* disrupts *Rhagoletis* monophyly in the mtDNA tree (Fig. 2) by virtue of its placement as a sister to the South American *Rhagoletis*. However, in neither case is there branch support for these relationships. *Zonosemata* species infest fruits of the genus *Solanum*, as do all of the Neotropical *Rhagoletis*, suggesting that they may have evolved from a common *Solanum*-infesting ancestor.

We will only be able to answer questions about the taxonomic status of *Rhagoletis* within the Carpomyina by more thorough sampling of taxa. Both *Carpomya* spp. and *Zonosemata* spp. are underrepresented in our phylogenetic analyses of Carpomyina, and it would be worthwhile to focus energy on the inclusion of members of these genera in future studies. It may be that *Rhagoletis* will need to be reclassified within the Carpomyina by synonymy with *Carpomya*. Unfortunately, the junior *Rhagoletis* is both species-rich and economically important worldwide, while the senior *Carpomya* is not. Thus, we should have especially strong support for phylogenetic relationships used to effect taxonomic change, as the utility of the current classification would surely be compromised.

Why are the morphological and mitochondrial DNA data sets incongruent?

The incongruence length difference test that we conducted (Fig. 5) indicates that the morphological and mitochondrial DNA data contain significantly different phylogenetic signals. Several reasons have been proposed why two data sets might contain conflicting phylogenetic signals (Thornton and DeSalle, 2000). Among these are: randomness in the distribution of homoplasy across the two data partitions, long-branch attractions in one data partition and not the other, differential natural selection between the two partitions, and population level processes such as differential lineage sorting.

There is reason to think that differential lineage sorting may be operating in *Rhagoletis* spp., especially if host shifting constitutes the major mechanism of cladogenesis (Bush, 1966, 1969, 1992). Phylogenetic trees of Nearctic *Rhagoletis* species based on DNA sequences of nuclear

alleles yield different topologies depending on the locus used in the analysis (Feder et al., personal communication). The mtDNA trees, especially the neighbor-joining tree shown as an unrooted phylogram (Fig. 4), give the impression that the Nearctic *Rhagoletis* spp. underwent an adaptive radiation at some point in time. If a large, genetically diverse ancestral *Rhagoletis* population radiated into several new host plant environments, with little or no reduction in population size, the resulting species would have been subject to considerable genetic drift resulting in the random fixation of alleles in the resulting lineages. Moore (1995) pointed out that a particular gene tree is most likely to be incongruent with the species tree when internodes are short and broad (i.e., when effective population sizes are large).

Differential natural selection between the morphological and mtDNA data partitions is also a reasonable explanation for the observed incongruence. Morphological characters, such as wing and body color patterns, will be subject to very different selection regimes than will mtDNA genes. One feature emerging from our phylogenetic analysis is that some species groups, such as the *R. suavis* group, appear to have specialized on one or only a few closely related plant genera. The *R. suavis* group infests only *Juglans* spp., and the Neotropical *Rhagoletis* in the *R. nova* group infest only fruits of *Solanum* spp. These tephritid groups apparently evolved when an early ancestor shifted and subsequently radiated onto a previously unexploited host. Further analysis will be required to explain why some groups specialize within a host plant family, while others always shift to unrelated hosts during the course of speciation.

Future efforts should focus on increasing the sampling density within the subtribe to examine more completely any possible incongruencies between genetic and morphology-based estimates of phylogeny, and to clarify unresolved nodes.

ACKNOWLEDGMENTS

We thank Amnon Freidberg and his students and colleagues at Tel Aviv University for organizing and producing the 1st Tephritidiologist Conference. We also thank Vesna Gavrilovic, Joe Roethele and Jeff Feder for providing unpublished data, and George Weiblen for helpful discussions.

REFERENCES

Berlocher, S.H. 1981. A comparison of molecular and morphological data, and phenetic and cladistic methods, in the estimation of phylogeny in *Rhagoletis* (Diptera: Tephritidae). In: *Application of genetics and cytology in insect systematics and evolution* (M. W. Stock, ed.), University of Idaho, Moscow. 152 pp.

Berlocher, S.H. and Bush, G.L. 1982. An electrophoretic analysis of *Rhagoletis* (Diptera: Tephritidae) phylogeny. *Systematic Zoology* 31: 136-155.

Berlocher, S.H., McPheron, B.A., Feder, J.L. and Bush, G.L. 1993. Genetic differentiation at allozyme loci in the *Rhagoletis pomonella* (Diptera: Tephritidae) species complex. *Annals of the Entomological Society of America* 86: 716-727.

Bush, G.L. 1966. The taxonomy, cytology, and evolution of the genus *Rhagoletis* in North America (Diptera, Tephritidae). *Bulletin of the Museum of Comparative Zoology* 134: 431-562.

Bush, G.L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). *Evolution* 23: 237-251.

Bush, G.L. 1992. Host race formation and sympatric speciation in *Rhagoletis* fruit flies (Diptera: Tephritidae). *Psyche* 99: 335-357.

Carpenter J.M. 1988. Choosing among multiple equally parsimonious cladograms. *Cladistics* 4: 291-296.

Farris, J.S. 1969. A successive approximations approach to character weighting. *Systematic Zoology* 18: 374-385.

Farris, J.S. 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417-419.

Farris, J.S., Kallersjo M., Kluge A.M. and Bult C. 1995. Constructing a significance test for incongruence. *Systematic Biology* 44: 570-572.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

Foote, R.H. 1981. The genus *Rhagoletis* Loew south of the United States (Diptera: Tephritidae). USDA Technical Bulletin Number 1607, 75 pp.

Foote, R.H., Blanc, F.L. and Norrbom, A. L. 1993. *Handbook of the fruit flies (Diptera: Tephritidae) of America north of Mexico.* Cornell University Press, Ithaca, NY. xii + 571 pp.

Han, H.-Y. and McPheron, B.A. 1997. Molecular phylogenetic study of Tephritidae (Insecta: Diptera) using partial sequences of the mitochondrial 16S ribosomal DNA. *Molecular Phylogenetics and Evolution* 7: 17-32.

Jenkins, J. 1996. Systematics studies of *Rhagoletis* and related genera (Diptera: Tephritidae). Ph.D. thesis, Michigan State University. 184 pp.

Maddison, W.P. and Maddison, D.R. 1992. *MacClade: Analysis of phylogeny and character evolution. Version 3.0.* Sinauer Associates, Sunderland, Massachusetts.

McAlpine, J.F. 1981. Morphology and terminology-adults. pp. 9-63. In: McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R., Wood, D.M., (coordinators), *Manual of Nearctic Diptera*, vol. 1. Agriculture Canada, Monograph No. 27, Ottawa.

McPheron, B.A. and Han, H.-Y. 1997. Phylogenetic analysis of North American *Rhagoletis* (Diptera: Tephritidae) and related genera using mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 7: 1-16.

Moore W.S. 1995. Inferring phylogenies from mtDNA variation: Mitochondrial-gene trees versus nuclear-gene trees. *Evolution* 49: 718-726.

Norrbom, A.L. 1994. New species and phylogenetic analysis of *Cryptodacus*, *Haywardina*, and *Rhagoletotrypeta* (Diptera: Tephritidae). *Insecta Mundi* 8: 37-65.

Norrbom, A.L. 1997. The genus *Carpomya* Costa (Diptera: Tephritidae): New synonymy, description of first American species, and phylogenetic analysis. *Proceedings of the Entomological Society of Washington* 99: 338-347.

Smith, J.J. and Bush, G.L. 1997. Phylogeny of the genus *Rhagoletis* (Diptera: Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxidase II. *Molecular Phylogenetics and Evolution* 7: 33-43.

Smith, J.J. and Bush, G.L. 1999. Phylogeny of Carpomyina, emphasizing relationships of the genus *Rhagoletis*. pp. 187-217. In: Aluja, M. and Norrbom, A.L. (eds.), *Fruit flies (Diptera: Tephritidae): Phylogeny and evolution of behavior*, CRC Press, Boca Raton, Florida.

Swofford, D.L. 2000. PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods).* Version 4. Sinauer Associates, Sunderland, Massachusetts.

Thornton J.W. and Desalle R. 2000. A new method to localize and test the significance of incongruence: Detecting domain shuffling in the nuclear receptor superfamily. *Systematic Biology* 49: 183-201.

White, I.M., Headrick, D.H., Norrbom, A.L. and Carroll, L.E. 1999. Glossary, pp. 881–924. In: Aluja, M. and Norrbom, A.L. (eds.), *Fruit flies (Diptera: Tephritidae): Phylogeny and Evolution of Behavior*, CRC Press, Boca Raton, Florida.

APPENDIX 1

Descriptions of morphological characters used in the parsimony analysis (from Jenkins, 1996; modified to conform with terminology of McAlpine (1981) and White *et al.* (1999))

Character number	Jenkins designation	Character description and states	Status in dataset
1	HEAD 5a	Flagellum rounded or angular dorsoapically and without detectable point (0); or flagellum more or less angular dorsoapically and with at least small dorsoapical point (1)	informative
2	HEAD 6	Distal half of arista bare or with few scattered rays(1); or arista uniformly rayed (0)	informative
3	HEAD 10	Facial ridge about as wide as or narrower than parafacial (0); or facial ridge decidedly wider than parafacial (1)	constant
4	HEAD 17	Genal seta concolorous (0) or not concolorous (1) to principle head setae (excluding gular, postocellar, and postocular)	informative
5	HEAD 18	Gular seta concolorous (0) or not concolorous (1) to principle head setae (excluding genal, postocellar, and postocular)	informative
6	HEAD 19	Postocellar seta concolorous (0) or not concolorous (1) to principle head setae (excluding genal, gular, and postocular)	informative
7	HEAD 20	Postocular seta concolorous (0) or not concolorous (1) to principle head setae (excluding genal, gular, and postocellar)	informative
8	HEAD 23a	Posterior orbital seta absent (1) or present (0)	informative
9	HEAD 25	Genal setae enlarged, numerous, or both (1); or genal setae not enlarged or unusually numerous (0)	constant
10	HEAD 26	Male with frontal setae pointed and similar in size to frontal setae of female (0); or frontal setae of male blunt and larger than frontal setae of female (1)	constant
11	THOR 5	Ground color of scutum yellowish (0); or ground color black or brownish (1)	informative
12	THOR 6b	Integument of scutum with *Carpomya*-like pattern (1); with whitish or yellowish medial stripe or prescutellar spot (2); or more or lesss uniformly pigmented or with intraspecifically variable pattern (0)	informative
13	THOR 10	Disc of scutum with microtrichia (0); or disc lacking microtrichia, scutum with peripheral microtrichia only	informative
14	THOR 12	Disc of scutum with setulae of uniform color (0); or disc of scutum with mixture of light and dark setulae	informative
15	THOR 14a	Supra-alar area with unmodified microtrichia (0); or supra-alar area with black, velvety microtrichia (1)	informative
16	THOR 16	Mediotergite with simple microtrichia (0); or mediotergite with pollenose microtrichia	uninform-ative
17	THOR 18	Halter wholly yellowish or brownish (0); or halter with stem yellowish and knob dark brown or black (1)	informative
18	THOR 23	Lateral scapular seta concolorous (0) or not concolorous (1) to principle thoracic setae (excluding presutural acrostichal, and proepisternal setae)	informative
19	THOR 24	Proepisternal setae concolorous (0) or not concolorous (1) to principle thoracic setae (excluding lateral scapular, and presutural acrostichal setae)	informative
20	THOR 31	Bare spots at medial ends of transverse suture and base of postsutural dorsocentral seta (1); or transverse suture and base of postsutural dorsocentral seta without bare spots (0)	uninform-ative
21	WING 2	Radial-medial band present (0) or radial-medial band absent (1)	informative

Appendix 1 (cont.)

Character number	Jenkins designation	Character description and states	Status in dataset
22	WING 3b	Subcostal band not crossing crossvein R-M (0) or subcostal band crossing crossvein R-M (1)	uninformative
23	WING 10	At least proximal hairs of fringe of dorsal calypter dark brownish or black (0); or all hairs of fringe of dorsal calypter whitish (1)	informative
24	WING 16	Cell br within subcostal band with hyaline spot (1); or cell br within subcostal band entirely pigmented or part of larger hyaline area (0)	constant
25	WING 18	Wing pattern with subcostal, radial-medial, and subapical bands fused anteriorly, and humeral and subcostal bands fused posteriorly (1); or wing pattern with one or more of these bands not fused as described (0)	informative
26	WING 19	Wing pattern with humeral, subcostal, and subapical bands free posteriorly, radial-medial band absent, and apical band with posterodistal corner of anterior arm well ahead of vein M (1); or wing pattern otherwise (0)	informative
27	LEGS 2a	Hindfemur wholly yellowish (0) or infuscated (1)	informative
28	LEGS 3	Tarsomere 4 or 5 or both same color as rest of tarsus (usually yellowish) (0); or darker than basal segments (1)	informative
29	LEGS 4	Midtibia with distinct posterodorsal row of setae (0); or midtibia without distinct posterodorsal row of setae (1)	informative
30	LEGS 5	Hindtibia with distinct anterodorsal row of setae (0); or hindtibia without distinct anterodorsal row of setae (1)	constant
31	LEGS 6	Midfemur or hindfemur or both with enlarged setae ventrally (1); or both femora with setae not enlarged (0)	uninformative
32	LEGS 7	Males with anteroventral row of setae on forefemur enlarged (1); or anteroventral row with setae on forefemur normal, not enlarged (0)	informative
33	LEGS 8	Fifth tarsomere relatively small, cylindrical, about twice as long as maximum diameter (1); or fifth tarsomere larger, flattened, less than twice as long as maximum diameter (0)	uninformative
34	ABDO 4	Ground color or terga yellowish (0) or brownish to black (1)	informative
35	ABDO 6	Excluding tergite 1, one or more terga with bands of light and dark colored setae (1); or setal color of terga uniform (0)	informative
36	MALE 8	Sternum 7 of male with polygonal sculpturing (1); or sternum 7 of male without sculpturing (0)	uninformative
37	MALE 12	Basiphallic vesica present (1); or basiphallic vesica absent (0)	informative
38	MALE 13	Ejaculatory apodeme with distal edge flared (1); or ejaculatory apodeme with edge coplanar with blade of apodeme (0)	informative
39	MALE 15	Dorsal portion of epandrium produced posteriorly well beyond base of surstyli, angle formed by posterior edge of epandrium ventral to proctiger and long axis of surstyli decidedly less than 90º (1); or dorsal portion of epandrium not markedly produced posteriorly, angle formed by posterior edge of epandrium ventral to proctiger and long axis of surstyli about 90º or more (0)	uninformative
40	MALE 22	Hypandrial sac lined with numerous heavily sclerotized denticles (1); or hypandrial sac not lined with denticles, or intrahypandrial membrane not forming sac (0)	informative

Appendix A (cont.)

Character number	Jenkins designation	Character description and states	Status in dataset
41	MALE 29E	Glans with subapical lobe trumpet-shaped (0); with elongate lobe or flap (1); or with pair of large apical hooks (2)	informative
42	MALE 31	Bacilliform sclerites with dorsal keel, at least distally (1); or bacilliform sclerites rounded dorsally and without keel (0)	informative
43	MALE 36	Microtrichia present on base of lateral surstylus anteriorly (1); or base of lateral surstylus bare anteriorly (0)	informative
44	MALE 37	Membrane connecting bacilliform sclerites to lateral surstylus with microtrichia present (0); or membrane connecting bacilliform sclerites to lateral surstylus bare (1)	uninformative
45	MALE 39a	Epandrium with numerous, evenly distributed microtrichia (1); or epandrium without microtrichia or at most with few patchy ones (0)	informative
46	MALE 40	Oviscape with one or more setae (0); or oviscape with only setulae or bare (1)	informative
47	MALE 46a	Parameral sheath of distiphallus with polygonal sculpturing (0); or parameral sheath of distiphallus without polygonal sculpturing (1)	informative
48	MALE 47	Tip of lateral surstylus with cluster of long setae (1); or with setae shorter and not forming cluster (0)	informative
49	MALE 49	Lateral surstylus with *Carpomya*-like setae distally (1); or lateral surstylus with normal setae (0)	uninformative
50	MALE 50	Hypandrial apodeme present (0); Hypandrial apodeme absent (1)	informative
51	MALE 51a	Lateral surstylus with anterior lobe only (0); lateral surstylus with anterior and posterior lobes (1); or lateral surstylus with anterior, medial, and posterior lobes (2)	informative
52	MALE 55	Right pregonite deflected ventrally (0); or right and left pregonites even (1)	constant
53	MALE 56b	Acrophallus present (1); or acrophallus absent (0) Medial	informative
54	MALE 60a	prensiseta on large tubercle that places it decidedly distal of lateral prensiseta (1); or medial and lateral prensisetae at about same level (0)	constant
55	MALE 61	Hypoproct entire (0); or hypoproct divided (1)	uninformative
56	MALE 62	Hypoproct extending dorsally for most or all of height of proctiger (1); or hypoproct extending dorsally for less than half height of proctiger, if at all (0)	constant
57	MALE 66a	Medial and lateral prensisetae similar in size (0); medial prensiseta larger than lateral prensiseta (1); or medial prensiseta smaller than lateral prensiseta (2)	uninformative
58	MALE 69	Anterolateral corner of bacilliform sclerites forming lobes (0); or anterolateral corner of bacilliform sclerites not forming lobes (1)	uninformative
59	MALE 70a	Apex of glans enclosed by parameral sheath (1); or distal portion of aedeagus not enclosed by parameral sheath (0)	informative
60	MALE 71	Basiphallus with membranous ventral keels (1); or basiphallus without membranous ventral keels (0)	constant
61	MALE 75	Vesica contiguous with phallotheca (1); or vesica free distally (0)	uninformative
62	MALE 76a	Subapical distiphallic lobe bare (0); with numerous	informative

Appendix 1 (cont.)

Character number	Jenkins designation	Character description and states	Status in dataset
		sclerotized denticles (1); microtrichose (2); fimbriate without supernumerary lobe (3); or fimbriate with supernumerary lobe (4)	
63	FEML 3	Total number of spermathecae three (0); total number of spermathecae two (1); or total number of spermathecae four (2)	informative
64	FEML 5	Spermathecae cylindrical (0); or spermathecae globular (1)	informative
65	FEML 8	Number of spermathecal ducts: 3 (0); 2 (1), or 4 (2) Eversible	informative
66	FEML 11	membrane of ovipositor about as long as segment 8 (1);	uninform ative
		or eversible membrane distinctly longer than segment 8 (0)	
67	FEML 12	Eversible membrane of ovipositor with microtrichia proximally (1); or eversible membrane without microtrichia (0)	informative
68	FEML 13a	Denticles on eversible membrane of ovipositor near segment 8 with single point (0); or denticles near segment 8 with multiple points (1)	informative
69	FEML 15	Large discal denticles on ventral surface of eversible membrane of ovipositor triangular and with single point (0); or large discal denticles on ventral surface of eversible membrane squarish and irregular apically (1)	constant
70	FEML 18	Segment 8 constricted at base (1); or segment 8 not constricted basally (0)	constant
71	FEML 19	Segment 8 with tip laterally flattened (1); or segment 8 with tip dorsoventrally flattened (0)	constant
72	FEML 22a	Segment 8 with microtrichia or denticles or both around cloaca (0); or cloaca glabrous (1)	constant
73	FEML 24a	Tip of segment 8 with subapical points, projections or serrations (0); or tip of segment 8 with single, apical point (1)	informative
74	FEML 28	One spermatheca definitely smaller than other(s) (1); or spermathecae nearly same size (0)	informative
75	FEML 29	Spermathecal ducts definitely annulated and radiator	uninform ative
		hose-like (1); or spermathecal ducts smooth (0) Dorsal	
76	FEML 30	taeniae extend to segment 8 (1); or dorsal taeniae not reaching segment 8 (0)	constant
77	FEML 32	Ventrally, tip of oviscape with about 8-16 stout setae (1); or tip of oviscape with setae of normal size (0)	informative