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Analysis of host preference and geographical distribution of *Anastrepha suspensa* (Diptera: Tephritidae) using phylogenetic analyses of mitochondrial cytochrome oxidase I DNA sequence data

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Abstract

Anastrepha suspensa (Loew) is an economically important pest, restricted to the Greater Antilles and southern Florida. It infests a wide variety of hosts and is of quarantine importance in citrus, a multi-million dollar industry in Florida. The observed recent increase in citrus infested with *A. suspensa* in Florida has raised questions regarding host-specificity of certain populations and genetic diversity of the pest throughout its geographical distribution. Cytochrome oxidase I (COI) DNA sequence data was used to characterize the genetic diversity of *A. suspensa* from Florida and Caribbean populations reared from different host plants. Maximum likelihood and Bayesian phylogenetic methods were used to analyse COI data. Sequence variation among mitochondrial COI genes from 107 *A. suspensa* samples collected throughout Florida and the Caribbean ranged between 0 and 10% and placed all *A. suspensa* as a monophyletic group that united all *A. suspensa* in a clade sister to a Central American group of the *A. fraterculus* paraphyletic species complex. The most likely tree of the COI locus indicated that COI sequence variation was too low to provide resolution at the subspecies level, therefore monophyletic groups based on host-plant use, geography (Florida, Jamaica, Cayman Islands, Puerto Rico or Dominican Republic) or population sampled are not supported. This result indicates that either no population segregation has occurred based on these biological or geographical distinctions and that this is a generalist, polyphagous invasive genotype. Alternatively, if populations are distinct, the segregation event was more recent than can be distinguished based on COI sequence variation.

Keywords: *Anastrepha suspensa*, Caribbean fruit fly, *fraterculus*, genetic variation, fruit flies

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Introduction

The genus *Anastrepha* Schiner (Diptera: Tephritidae) contains 18 species groups (197 described species) defined by morphology and host plant use (Aluja *et al*., 1999). Species

of *Anastrepha* are endemic to subtropical and tropical areas in the Americas (Stone, 1942; Aluja, 1994), and infest a wide variety of economically important hosts (Aluja, 1994). *Anastrepha* is a monophyletic genus based on both morphology (Norrbom *et al*., 1999) and 16s rRNA mitochondrial DNA sequence data (McPheron *et al*., 1999). Morgante *et al*. (1980), however, observed discrepancies between molecular data (protein electrophoresis) and morphological data used for inferring evolutionary relationships within *Anastrepha*, and most recently, molecular phylogeny using the nuclear gene *period* suggests *Anastrepha* is paraphyletic in placement with respect to *Toxotrypana* (a closely related genus) (Barr *et al*., 2005). Phylogenetic placement and systematic relationships of species within *Anastrepha* has been the focus of evolutionary biologist for decades. Several species appear to be paraphyletic including *A. fracterculus* (Wiedemann) and *A. obliqua* Macquart (Smith-Caldas *et al*., 2001), while relationships of *A. suspensa* (Loew) have not been investigated and current placement of *A. suspensa* within *Anastrepha* is based on a small number of individuals.

Anastrepha suspensa, the Caribbean fruit fly, is an economically important pest of many tropical and subtropical fruits of Florida and the Greater Antilles. This species is a member of the *fracterculus* species group, which includes 29 species (Steck, 1991; McPheron *et al*., 1999; Norrbom *et al*., 1999; Smith-Caldas *et al*., 2001; Barr *et al*., 2005) and this species group infests a diverse group of hosts, some of which are economically important (Aluja, 1994; Norrbom *et al*., 1999). The *fracterculus* species group is monophyletic based on both molecular (McPheron *et al*., 1999; Norrbom *et al*., 1999; Smith-Caldas *et al*., 2001; Barr *et al*., 2005) and morphological data (Norrbom *et al*., 1999). Several studies have focused on evolutionary relationships within the *fraterculus* species group (Steck, 1991; Smith-Caldas *et al*., 2001) due to the economic importance of this species in South America. The most recent phylogenetic analyses of *A. fraterculus* (the species for which the *fraterculus* species group is named) were based on COI sequence data from the mitochondrial genome and revealed it is a paraphyletic species and thus is considered a species complex (Smith-Caldas *et al*., 2001). Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) of mitochondrial DNA (Steck & Sheppard, 1993) and protein electrophoresis (Morgante *et al*., 1980; Steck, 1991) data show high levels of diversity within *A. fraterculus*. A consistent finding for all studies involving *Anastrepha* phylogenetics is members of the *A. fraterculus* species complex are most closely related to *A. suspensa* (Barr *et al*., 2005; McPheron *et al*., 1999; Smith-Caldas *et al*., 2001), not *A. ludens* (Loew) as previously reported (Weems, 1965; Weems *et al*., 2001).

Questions concerning population differentiation due to host plant preferences and other factors, such as multiple introductions, have been raised. For example, Weems (1965) speculated that a large outbreak of *A. suspensa* in south Florida during 1965 was comprised of flies with population dynamics and host plants more similar to those of a Puerto Rican strain of *A. suspensa* than to those of a population of *A. suspensa* that once occurred in the Florida Keys. The native population of *A. suspensa* apparently died out some time after 1936, not a single specimen was collected anywhere in Florida in the field after 1936 until 1956 (Weems, 1965). The 1965 outbreak was thus thought to be a new introduction of *A. suspensa* from Puerto Rico (Weems, 1965). This conclusion was based on field host experiments where *A. suspensa* was infesting mangoes and in one case a sour orange, which was similar to flies in Puerto Rico. Whether that introduction was the parent source for all *A. suspensa* populations currently found in Florida or if additional introductions from Puerto Rico or other Caribbean areas have occurred is not known. No host shifts have been noted in the literature for Florida populations of *A. suspensa*, but changes in plant diversity due to urbanization and agricultural practices may have led, or could lead, to new strains.

Genetic studies have proven to be very useful in determining the genetic differences between populations of *A. suspensa*. For example, Schnell *et al*. (1996) showed the utility of random amplified polymorphic DNA markers in identifying highly inbred colonies of *A. suspensa* and Heath *et al*. (2002) generated a mitochondrial DNA restriction map that was found to be polymorphic among individuals in highly inbred colonies and a feral population.

Mitochondrial DNA sequence data from the 3-prime COI region have proven useful for phylogenetic studies of Tephritidae (Han & McPheron, 1999), specifically *Rhagoletis pomonella* (Walsh) (Feder *et al*., 2003), *Bactrocera dorsalis* (Hendel) (Shi *et al*., 2005) and also for distinguishing species within the complexes of *Bactrocera tau* (Walker) (Jamnongluk *et al*., 2003) and *A. fraterculus* (the sister group to *A. suspensa*) (Smith-Caldas *et al*., 2001). Therefore, COI was chosen for this investigation into the genetic diversity of *A. suspensa* in Florida and parts of the Caribbean that may have arisen through host shifts or geographic expansion. Current molecular phylogenetic work on *A. suspensa* COI sequence variation is very limited in sample size and sample collection location (Smith-Caldas *et al*., 2001). The purpose of the present study was to determine if there was any relationship between *A. suspensa* collected from a variety of hosts and geographical locations throughout its current distribution in Florida and the Caribbean. Specimens collected in Florida during 1935 and 1965 and in Puerto Rico and other Caribbean areas during 2004 and 2005 were included in the analyses.

Materials and methods

Outgroup determination

To determine the phylogenetic placement of *A. suspensa* within the *A. fraterculus* group an alignment of COI sequence data including 12 species within the group and two species (*A. striata* Schiner and *A. serpentina* (Wiedemann)) outside the *fraterculus* group was generated. This alignment consisted of all sequences used in Smith-Caldas (2001) GenBank (accession numbers: AF420611–AF420655) and all samples listed in table 1. A phylogenetic analysis of the data set followed the procedures listed below in the phylogenetic analyses section.

Selection and collection of A. suspensa *populations*

All flies were reared from fruit of the specified host plants unless otherwise noted in table 1. *Anastrepha suspensa* museum specimens from 1935 and 1965 were provided by Dr Gary Steck (Florida State Collection of Arthropods, Gainesville, Florida). In collaboration with the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, and their insect monitoring programme, *A. suspensa* was obtained from various populations in central

Table 1. Collections of *Anastrepha* species used in generating fig. 1.

Species	Location	Host	Host (common name)	Population number	Accession number
A. suspensa	Vero Beach, FL	*Eugenia uniflora* L.	Surinam cherry	1	AY944905 AY944901 AY945001 AY944944 AY944991 AY944915 AY944996 AY944990 AY944993 AY944982 AY944928 AY944998 AY944933 AY944914 AY944921
A. suspensa	Vero Beach, FL	*Eugenia uniflora* L.	Surinam cherry	2	AY944942
A. suspensa	Fort Pierce, FL	*Citrus* × *paradisi* Macfad. (pro sp.)	Pink grapefruit	3	AY944898 AY944939 AY944956 AY944985 AY944917
A. suspensa	Vero Beach, FL	*Psidium guajava* L.	Guava	4	AY945004 AY944997 AY944981 AY944979
A. suspensa	Vero Beach, FL	*Eriobotrya japonica* (Thunb.) Lindl.	Loquat	5	AY944994 AY944995 AY944951 AY944945 AY944961 AY944967 AY944932 AY944936 AY944960
A. suspensa	Vero Beach, FL	*Eriobotrya japonica* (Thunb.) Lindl.	Loquat	6	AY944920
A. suspensa	Miami, FL	*Eugenia uniflora* L.	Surinam cherry	7	AY945003 AY944943 AY944955 AY944938 AY944929 AY944975 AY944966
A. suspensa	Fort Pierce, FL	*Citrus reticulata* Blanco × *Citrus sinensis* L.	Murcott	8	AY944978 AY944908
A. suspensa	Fort Pierce, FL	*Citrus paradisi* Macfad.	Grapefruit	9	AY944946 AY944974 AY944980 AY944953 AY944959 AY944947 AY944973 AY944931 AY944925
A. suspensa	Miami, FL	*Psidium cattleianum* Sabine	Guava	10	AY944937 AY944999 AY944954 AY944923 AY944924
A. obliqua	Jamaica	*Mangifera indica* L.	Mango	11	AY945061 AY945063 AY945057 AY945055

Table 1. Continued.

Species	Location	Host	Host (common name)	Population number	Accession number
A. obliqua (Cont.)					AY945053 AY945066 AY945071 AY945062 AY945054 AY945056 AY945064 AY945059 AY945069 AY945067 AY945070
A. suspensa	Vero Beach, FL	*Severinia buxifolia* (Poir.) Ten.	Box-orange	12	AY944968
A. suspensa	Miami, FL	*Manilkara zapota* (L.) P. Royen	Sapodilla	13	AY944927 AY944987 AY944965 AY944926 AY944935
A. ludens	USDA, Mission, TX	Laboratory colony	Laboratory colony	14	AY945008 AY945005 AY945007 AY945006 AY945009
A. suspensa	Fort Pierce, FL	*Psidium cattleianum* Sabine	Guava	15	AY944984
A. suspensa	Jamaica	*Mangifera indica* L.	Mango	16	AY944934
A. suspensa	Cayman Islands	*Swietenia mahagoni* Jacq.	Mahogany	17	AY944962
A. suspensa	Stuart, FL	*Eugenia uniflora* L.	Surinam cherry	18	AY944957 AY944949
A. suspensa	Fort Pierce, FL	*Psidium cattleianum* Sabine	Guava	19	AY944941
A. suspensa	Dominican Republic	Unknown	Unknown	20	AY944922 AY944988 AY944964 AY944906 AY944911 AY944952 AY944903 AY944989 AY944930
A. suspensa	Miami, FL	*Terminalia catappa* L.	Tropical almond	21	AY944904 AY944976 AY944902 AY944983 AY944907 AY944971
A. suspensa	Puerto Rico	*Psidium guajava* L.	Guava	22	AY944899 AY944977 AY944913 AY944992 AY945002 AY944919 AY944900 AY944910
A. suspensa	Miami Springs, FL	1965	Mango (McPahil)	24	AY944970
A. suspensa	Miami Springs, FL	1965	Guava (McPhail)	25	AY944909
A. suspensa	Key West, FL	1935	Unknown	32	AY944950
A. suspensa	St Lucie, FL	Unknown	Unknown	37	AY944918 AY944912
A. suspensa	Naples, FL	× *Citrofortunella mitis* J. Ingram & H.E. Moore	Calamondin	42	AY944986 AY944969

Table 1. Continued.

Species	Location	Host	Host (common name)	Population number	Accession number
A. obliqua	Puerto Rico	*Spondias lutea* L.	Jobo	43	AY945068 AY945060 AY945058 AY945065
A. suspensa	Puerto Rico	*Psidium guajava* L.	Guava	44	AY944972 AY945000 AY944948 AY944940

Population number corresponds to the first number listed after the species name in the phylogeny (figs 2 and 3). No two populations were found at the same site, for example, populations 1 and 2 are both from Surinam cherry in Vero Beach, Florida but were found at different locations in Vero Beach, Florida.

and southern Florida during 2004 and 2005. The presence of populations was determined by occurrence of *A. suspensa* in McPhail traps (Riherd & Jenkins, 1996). When flies were observed in traps, fruit from host plants in the vicinity of the traps were collected and stored in screen-enclosed buckets containing either vermiculite or sand. Flies that emerged (on average after three weeks) and confirmed as *A. suspensa* were then placed directly into 95% ethanol and stored at −20°C for further processing. Collaborators provided ethanol-preserved specimens from Florida, Puerto Rico, Jamaica, Cayman Islands and Dominican Republic. Dr Gary Steck (Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida) confirmed species identification of all specimens. *Anastrepha fraterculus* sequences most closely related to *A. suspensa* as determined by Smith-Caldas *et al*. (2001) were included as the outgroup.

DNA extraction, amplification and sequencing

Total DNA was extracted from individual flies using one of two methods: (i) BioRad AquaPure Genomic DNA Kit (732-6340) following the standard procedure 'A' protocol for extraction of DNA from tissue; or (ii) Cartagen's (www.cartagen.com) rapid homogenization for plant leaf DNA amplification (catalogue no. 20700-500, lot no. 08180400134). The DNA was amplified using COI primers C1-J-2183 (5′ CAACATTTATTTTGATTTTTTGG 3′) and TL2-N-3014 (5′ TCCAATGGACTAATCTGCCATATTA 3′) (Simon *et al*., 1994). The 30 µl PCR reactions used a thermal regime of 94°C for 2 min followed by 35 cycles of 30 s at 94°C, 30 s at 53°C, 1 min at 72°C, and 10 min at 72°C in a MJ Research PTC-200 Peltier thermal cycler. PCR products were cleaned prior to sequencing using montage PCR filter units (Millipore catalogue no. UFC7PCR50). Bidirectional sequencing was performed using the PCR primers and BigDye® Terminator Cycle Sequencing Kit Version 3.1. Sequence product analysis was conducted on an Applied Biosystems 3730xl DNA Analyser. Sequence fragments were assembled with Sequencher® version 4.2 (Gene Codes Corporation, 2004) and aligned using ClustalX (Thompson *et al*., 1997). Minor alignment issues were corrected using Se-Al (Rambaut, 2000).

Phylogenetic analyses

Molecular data were evaluated using maximum likelihood (ML) and Bayesian methods. ML analyses were performed using PAUP* (Swofford, 2003) and Bayesian analyses using MrBayes version 3.0b4 (Huelsenbeck & Ronquist, 2003). Prior to likelihood or Bayesian analysis the best-fit model of evolution was determined using Modeltest 3.6 (Posada & Crandall, 1998). For a review of models of molecular evolution see Swofford *et al*. (1996).

All populations listed in table 1 were included in the phylogenetic analyses. Heuristic searches with ten random addition sequence replicates and tree-bisection-reconstruction (TBR) branch swapping were performed for all ML estimates. ML estimates of the COI phylogeny were obtained using the Hasegawa Kishino Yano (HKY) model of molecular evolution (variable base frequencies, variable transition and transversion frequencies) with invariable (I) sites and gamma (G) distributed site-to-site variation (G)− HKY+I+G. (Felsenstein, 1981; Hasegawa *et al*., 1985; Swofford *et al*., 1996).

ML bootstrap analyses of 100 replicates were performed using a heuristic search with ten random addition sequence replicates and TBR branch swapping. A Bayesian approach was also used to assess branch support because of its easy interpretation of results, its ability to incorporate prior information (uniform in our case) (Huelsenbeck & Ronquist, 2001) and some computational advantages (Larget & Simon, 1999). MrBayes (Huelsenbeck & Ronquist, 2001) employs Markov Chain Monte Carlo (MCMC) to approximate the posterior probabilities of phylogenies (Metropolis *et al*., 1953; Hastings, 1970; Green, 1995). The model of evolution used for Bayesian analyses of COI data was HKY+I+G (determined by ModelTest 3.6). MrBayes was run with four chains from ten different starting points. All runs were done for 1 million generations and trees were sampled every 100 generations. All runs reached a plateau in likelihood score, which indicated the Markov-Chain Monte Carlo (MCMC) chains converged. To determine the number of trees to discard due to the burn-in phase, tree likelihood versus number of generations was plotted; trees recovered before the run reached stationarity were discarded. All trees saved from all ten runs were summarized in PAUP* (see MrBayes manual) and the posterior probabilities were recorded on the maximum likelihood tree.

Genetic identification of A. suspensa

MacClade 4.0 (Maddison & Maddison, 2000) was used to identify positions in the 806 base pair alignment that were unique to *A. suspensa* (synapomorphic character). The

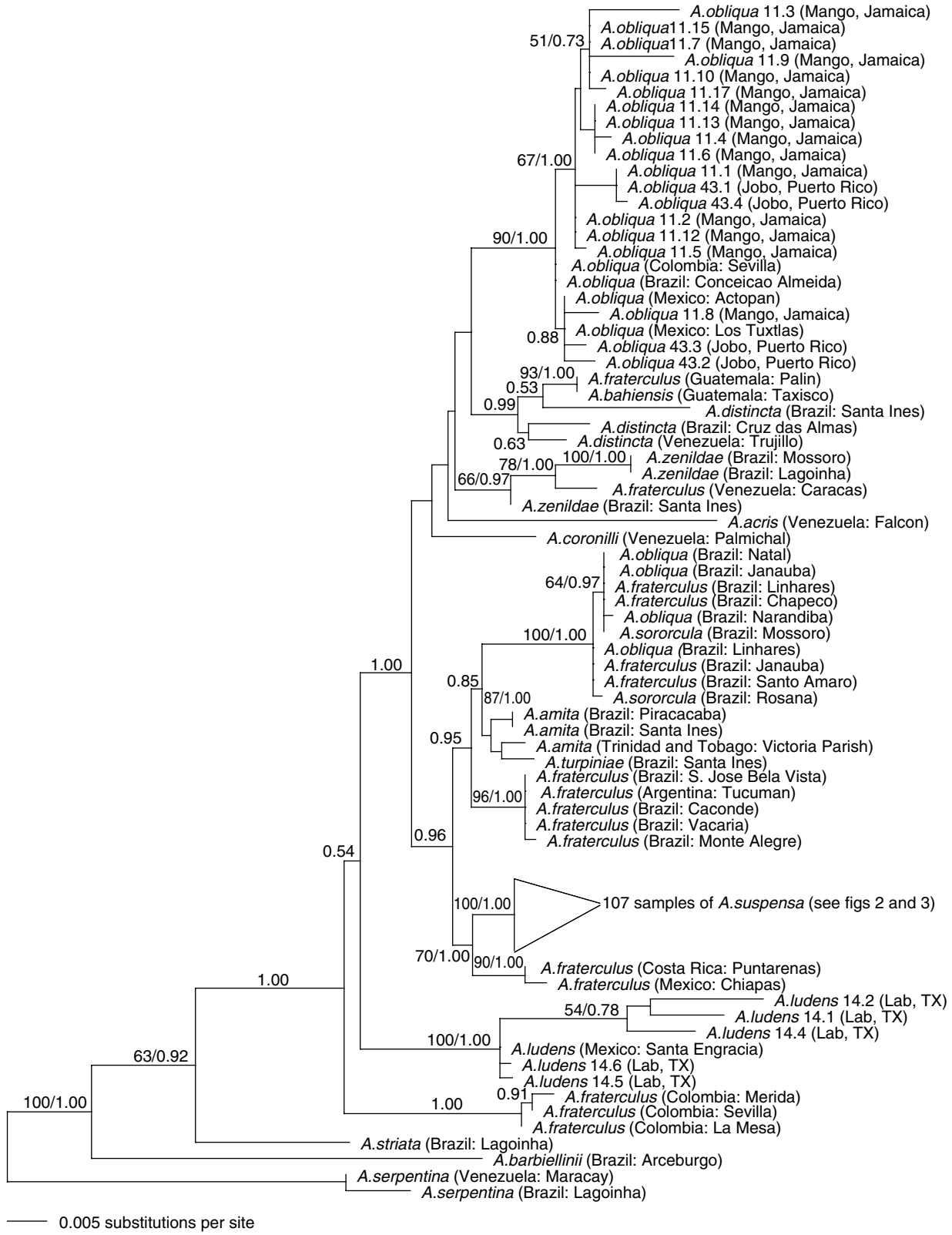

Fig. 1. Phylogram of the maximum likelihood tree generated using COI data and the GTR+G model of molecular evolution ($-\ln = -3723.37$, gamma shape = 0.2281) for all included *Anastrepha* species. A Bayesian analysis was run under the GTR+G model for 1 million generations and 8000 trees were used to assess the posterior probabilities of the nodes (2000 trees discarded due to burn-in).

alignment generated using ClustalX was used along with the most likely tree generated using PAUP*. In MacClade 4.0 the 'trace all states' and 'trace all changes display' were used and set to 'show bar for each change'. This identified each position in the alignment that was unique to certain groups found in the phylogenetic tree.

Constraint tree analyses

Monophyly of *A. suspensa* from citrus, the Caribbean, and Miami, Florida populations was tested using constraint tree analysis. For example, to test the monophyly of *A. suspensa* from citrus, all *A. suspensa* samples from citrus (populations 3, 8, 9 and 12, table 1) were constrained to a monophyletic group and an ML search was conducted under a HKY + I + G model of molecular evolution (determined by Modeltest). The Kishino-Hasagawa (1989) and the Shimodaira-Hasagawa (1999) tests were used to determine if the ML tree with no constraint was significantly different than the tree generated with the constraint enforced. Both the Kishino-Hasagawa and the Shimodaira-Hasagawa settings were set to full-optimization with 1000 bootstrap replicates. All analyses were performed using PAUP*.

Results

Molecular data

The dataset that contained all species of the *A. fraterculus* group was 806 base pairs long with no invariable sites. The model of molecular evolution was the general time reversible model (GTR) + G (gamma distributed site-to-site variation). Uncorrected P distances among species of the *A. fraterculus* group ranged from 0.00 to 0.10. Empirical base frequencies were: A = 0.3173, C = 0.1663, G = 0.1452, T = 0.3712 and the ML estimate of the gamma shape parameter is 0.2281.

A second data set contained 107 specimens of *Anastrepha suspensa* and two specimens *A. fraterculus* from Costa Rica and Mexico. Gaps in the alignment were treated as missing data. There were 303 variable sites out of the 806 base pairs; 0.01% of the matrix was treated as missing. Uncorrected P distances among *A. suspensa* ranged from 0.00 to 0.14. Empirical base frequencies were A = 0.3150, C = 0.1727, G = 0.1634, T = 0.3488. The transition to transversion ratio was 0.6463 and the proportion of invariable sites was 0.5410. The ML estimate of the gamma shape parameter was 0.7106.

Phylogenetic analyses

The most recent phylogeny of the *A. fraterculus* group is shown in fig. 1. The relationships recovered are similar to that shown in Smith-Caldas *et al*. (2001). With the addition of more specimens of *A. suspensa*, *A. obliqua*, and *A. ludens* through this study, the node support values have increased throughout the tree. The placement of two species, *A. acris* (Stone) and *A. coronilli* (Carrejo & Gonzalez) remains unknown. Our samples of *A. ludens* group with the Smith-Caldas *et al*. (2001) specimen with high node support at the base of the phylogenetic tree. *Anastrepha obliqua* is still a paraphyletic species with our specimens from Jamaica and Puerto Rico grouping with one of the two clades that contained *A. obliqua* in Smith-Caldas *et al*. (2001). The sister group relationship of *A. suspensa* with two collections of *A. fracterculus* from Costa Rica and Mexico as originally described by Smith-Caldas *et al*. (2001) was supported with high posterior probability and ML bootstrap values (100/70) for all 107 *A. suspensa* samples.

Importantly, although *A. fraterculus* remained paraphyletic (see Smith-Caldas *et al*., 2001 for discussion) all collections of *A. suspensa* were monophyletic (figs 1–3) with both maximum likelihood bootstrap (99) and Bayesian posterior probability (1.00) support. Position 492 was identified as the only synapomorphic character in comparison of *A. suspensa* and the closely related *A. fraterculus* species (data not shown); all 107 *A. suspensa* have a cytosine (C) while all *A. fraterculus* (the sister group) has a thymine (T). The Bayesian analyses provided more clade support, 20 versus 4 ML bootstrap nodes (fig. 2). The backbone of the most likely phylogenetic tree (fig. 2) was not well supported with either maximum likelihood bootstrap values or Bayesian posterior probabilities. There are several groups at the tips of the tree that are well supported, for example, *A. suspensa* 20.5 (from Puerto Rico) and *A. suspensa* 3.5 (from Fort Pierce, Florida) form a monophyletic group with 100/1.00 ML bootstrap and Bayesian posterior probability (fig. 2). The short branch lengths (fig. 3) indicate very few base pair differences between the samples of *A. suspensa* included in the analyses (table 1). *Anastrepha suspensa* samples that were included from 1935 (*A. suspensa* 32.1) and 1965 (*A. suspensa* 24.1 and *A. suspensa* 25.1) did not form a monophyletic group (figs 2 and 3). The 1935 sample was placed in a clade with flies collected in Miami reared from *Psidium cattleianum* Sabine, guava (*A. suspensa* 10.5) and *Eugenia uniflora* L., Surinam cherry (*A. suspensa* 7.5). However, there was no ML bootstrap or Bayesian posterior probability support for the placement of the 1935 fly. One of the 1965 flies (*A. suspensa* 24.1) was found towards the root of the tree, while the other fly from 1965 was placed in a clade with a fly from Vero Beach (*A. suspensa* 1.10) and Miami (*A. suspensa* 21.6). In the later case, there was minimal (0.65) Bayesian posterior probability support for the group.

There were no monophyletic groups defined in fig. 2 based on geography, population, or host-plant use. For example, all the flies from Fort Pierce, Vero Beach and Miami, Florida were found throughout the phylogeny (figs 2 and 3). Caribbean samples were also found scattered throughout the phylogeny. Individuals in single populations did not form a monophyletic group, for example, population 22 consisted of flies isolated from a single population of guava from Puerto Rico and individuals from this population were found throughout the phylogeny (figs 2 and 3). There were three populations of flies reared from fruit in the citrus family (Rutaceae), and none of them grouped together.

Constraint tree analyses

Results from the Kishino-Hasagawa (KH) and Shimodaira-Hasagawa (SH) tests (Kishino & Hasegawa, 1989;

Numbers are maximum likelihood bootstrap/Bayesian posterior probabilities. The *A. ludens* and *A. obliqua* collections with two numbers after the name correspond to the population numbers in table 1 and the second number is the individual number from a given population. All other data is from Smith-Caldas *et al*. (2001).

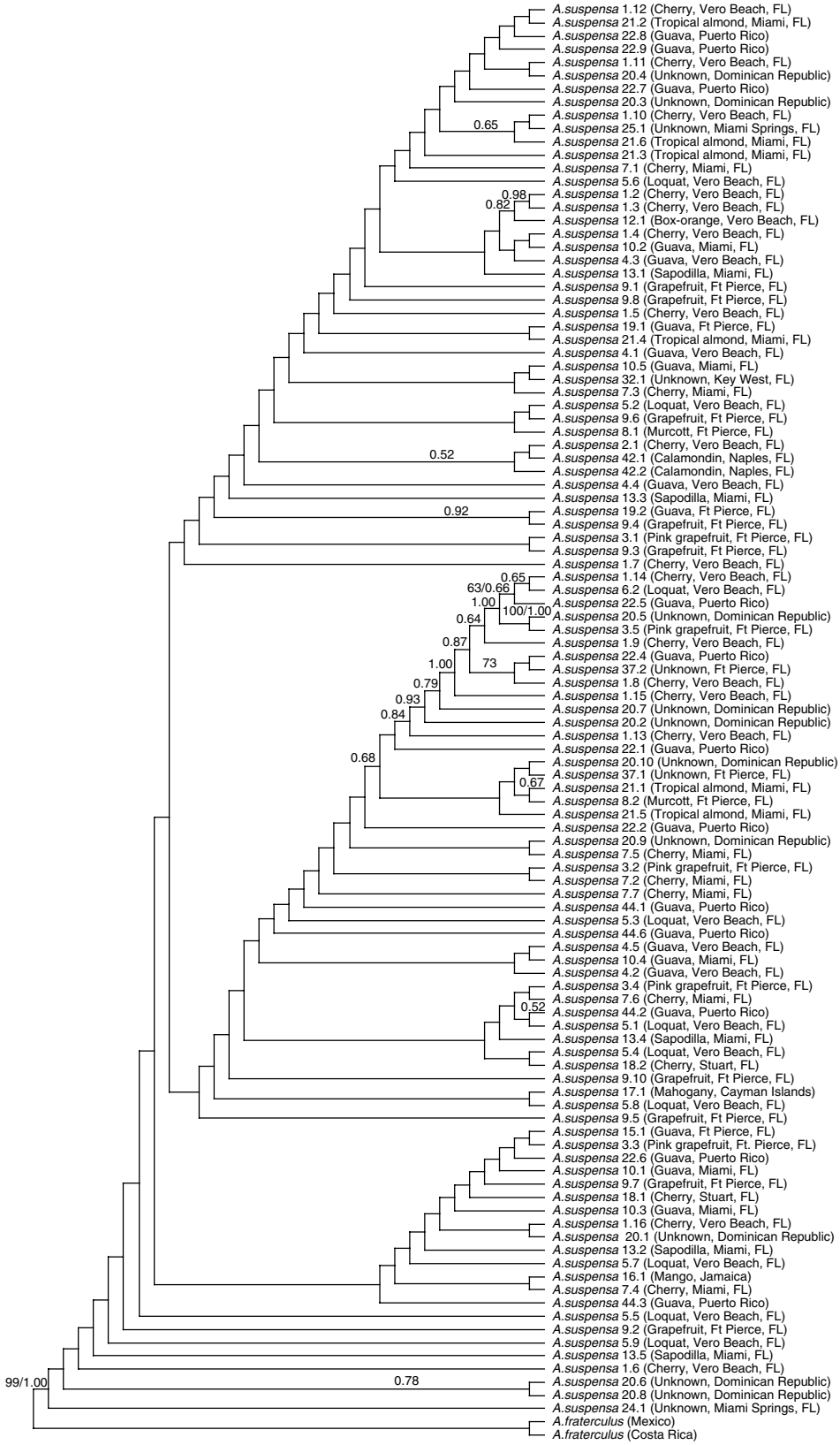

Fig. 2. For caption see opposite page.

Shimodaira & Hasegawa, 1999) showed significant difference between trees that contained *A. suspensa* from citrus, the Caribbean, and Miami, Florida each forced to be separate monophyletic groups (table 2). The differences between the most likely tree (fig. 2) and the constraint trees ranged from −1318.665511 (Caribbean) to −1337.71996 (citrus). This difference was great enough to require rejection of the hypothesis that variation in COI sequence data could be used to show population differences related to host preference or geographical location of *A. suspensa*.

Discussion

DNA sequences from the COI region showed *A. suspensa* is monophyletic, unlike its sister taxa, *A. fraterculus* (figs 1–3; Smith-Caldas *et al*., 2001). This is the first comparison of COI sequence data from *A. suspensa* taken throughout its geographic range, and this broad comparison offers support to the phylogenetic placement of *A. suspensa* that was previously determined from a single population sample (Smith-Caldas *et al*., 2001).

The maintenance of the monophyletic nature of *A. suspensa* populations, compared to some other tephritid species, could be supported by differences in habitat, behaviour and/or the time required for lineage sorting. Firstly, there were no geographic patterns observed in our phylogeny for *A. suspensa* generated using COI sequence data. Samples from the Caribbean (population 16 – Jamaica, population 17 – Cayman Islands, populations 20 and 44 – Puerto Rico) did not form separate monophyletic groups. There were no ML bootstrap or Bayesian probabilities to support the lack of geographic patterns, but the constraint tree analysis (table 2) illustrated that a tree with the Caribbean populations constrained to be a monophyletic group was significantly different from the best-fit tree (fig. 2). In contrast, *A. fraterculus* from South America appears geographically paraphyletic. Steck (1991), using isozyme data, and Smith-Caldas *et al*. (2001), using COI DNA sequence data, suggest that habitat differences found throughout its range of diverse elevational and climatic environments might explain this. Steck (1991) found two populations in close proximity in the Venezuelan lowland with very strong allelic frequency differences. These allelic differences could be explained as the result of natural selection and local adaptation of the two populations having very little to no gene flow between them because of elevational and climatic differences (Steck, 1991). These same patterns were observed in the phylogeny generated with COI data (Smith-Caldas *et al*., 2001), where three included Andean populations formed a strongly supported, highly divergent clade at the base of their *fraterculus* species complex tree. The current distribution of *A. suspensa* does not include as diverse elevational and climatic conditions as *A. fraterculus* being limited to the Greater Antilles and southern Florida. Secondly, samples of *A. suspensa* collected on the same fruit from different locations did not form monophyletic groups within the *A. suspensa* clade using COI DNA sequence data, and there was no significant ML bootstrap and Bayesian posterior probability support on the phylogeny regarding host specificity (fig. 2). Also, using the Kishino-Hasagawa (KH) and Shimodaira-Hasagawa (SH) tests (table 2), a phylogenetic tree constrained to group individuals from the same plant hosts was significantly different than the best-fit tree (fig. 2). These findings were in contrast to the results from COI data for another tephritid fruit fly, *Rhagoletis pomonella*, where *R. pomonella* collected from hawthorn and apples formed monophyletic clades respectively based on host. It was suggested that *R. pomonella* is a sibling species complex, which had diverged in sympatry by shifting (from hawthorn) and adapting to new host plants (apples) (Feder *et al*., 1995, 2003). Feder *et al*. (1995) suggest *R. pomonella* originally feeding on hawthorn escaped from predators and parasitoids when on apples, which could increase the suitability of apples as a host species. *Rhagoletis pomonella* from the United States and Mexico also formed separate monophyletic groups based on host-plant location (Feder *et al*., 2003)

Host availability was also an important factor when examining host-plant shifts. *Anastrepha suspensa* has different types of fruit available throughout the year, while *R. pomonella* does not. Increased fitness conferred by the ability to maintain population growth throughout the year by host shifting may prevent host preference from becoming an important population isolating factor, and therefore maintaining *A. suspensa* as a polyphagous species. In contrast, *R. pomonella* is thought to remain as a monophagous species (feeds on one host) due to a lack of suitable alternative hosts producing fruit throughout the year (Aluja *et al*., 1999).

Most monophagous species usually mate where the female oviposits (fruit or site of gall formation). In contrast, polyphagous species usually mate in aggregations on the foliage of host plants some distance from ovipositional sites (Burk, 1983). Maintenance of polyphagy in *A. suspensa* may therefore be due to a combination of host availability and mating system differences between *A. suspensa* and *R. pomonella*. Plant-host data was not provided in Smith-Caldas *et al*. (2001) for *A. fraterculus*, but host information might shed some light on the phylogeny of the paraphyletic *fraterculus* species complex.

There are considerable historical biogeography differences between the current distributions of *R. pomonella* and those of *A. suspensa* and *A. fraterculus* that could also influence the evolution of these groups of insects. It is hypothesized that an ancestral hawthorn-infesting *R. pomonella* population became divided into Mexican and North American populations approximately 1.57 million years ago (Feder *et al*., 2003) and has since shifted hosts to infest apple. The phylogenetic relationships among apparently isolated populations of *A. suspensa* may therefore not be reflected in the COI sequence variation because there has been less time for *A. suspensa* in Florida to accumulate genetic changes (1 mya- (Webb, 1990)).

Fig. 2. Cladogram of the maximum likelihood tree generated using COI data and the HKY + I + G model of evolution (−ln = −4805.198, ti/tv = 0.6463) for *Anastrepha suspensa* populations. A Bayesian analysis was run under the HKY + I + G model for 1 million generations and 7000 used to assess the posterior probabilities of the nodes trees (3000 were discarded due to burn-in). Numbers above the nodes are maximum likelihood bootstrap/Bayesian posterior probabilities. The first number after *A. suspensa* corresponds to the population numbers in table 1 and the second number is the individual number from a given population.

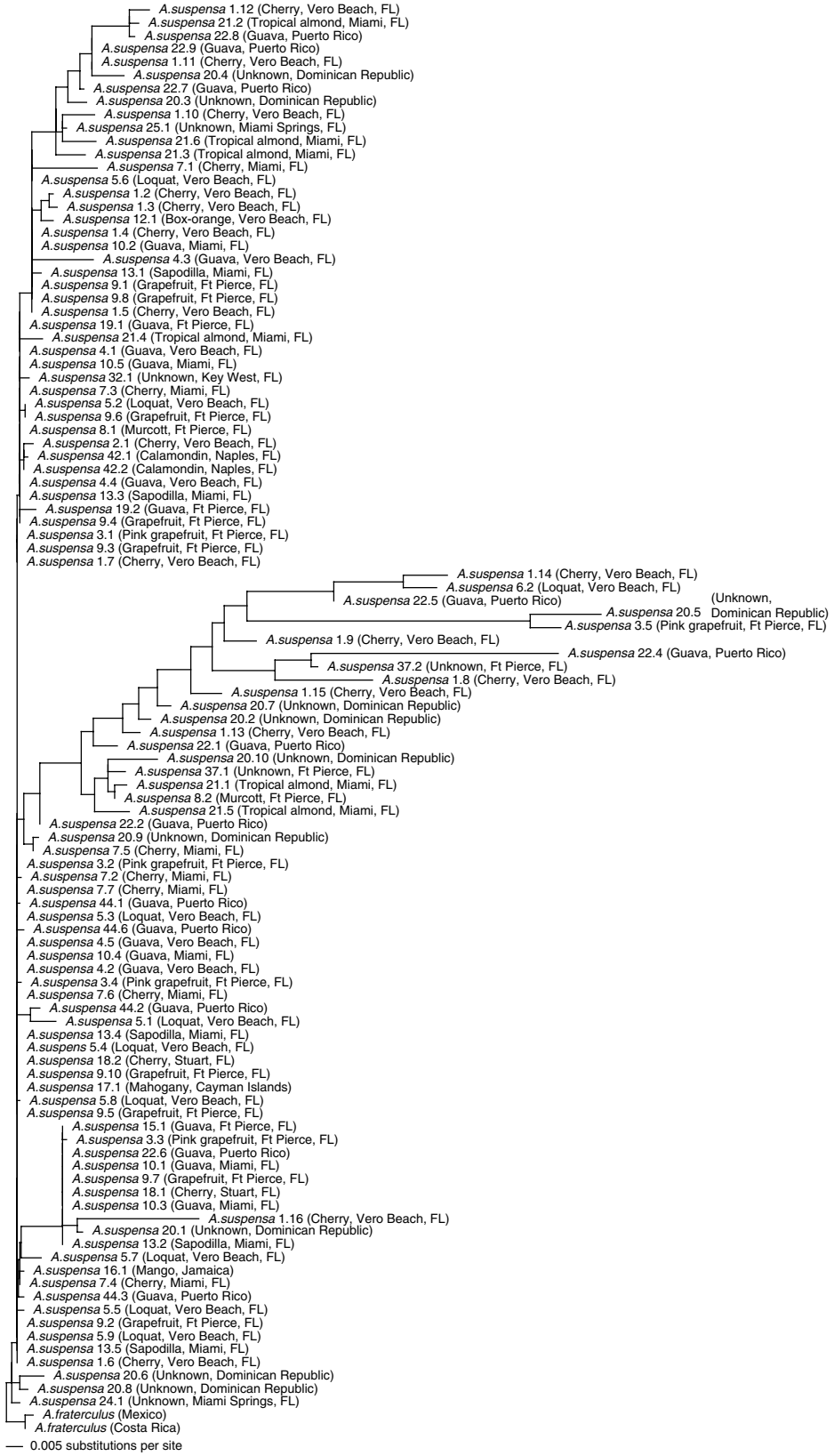

Fig. 3. For caption see opposite page.

Table 2. Topology test statistics for constraint tree analyses.

Constraint test	LN	Difference	KH-test *P*-value	SH-test *P*-value	Significant
Citrus					
Tree1	−4805.19809	Best	0.000	0.000	Yes
Tree2a	−6142.91804	1337.71996			
Caribbean					
Tree1	−4805.19809	Best	0.000	0.000	Yes
Tree2b	−6123.86360	1318.665511			
Miami					
Tree1	−4805.19809	Best	0.000	0.000	Yes
Tree2c	−6139.88043	1334.68234			

KH, Kishino-Hasegawa, SH, Shimodaira-Hasagawa (Kishino & Hasegawa, 1989; Shimodaira & Hasegawa, 1999). Tree 1 is the ML tree generated using PAUP*. Tree 2b contained all flies reared from citrus constrained to a monophyletic group. Tree 2b had all flies collected from the Caribbean constrained to a monophyletic group. Tree 2c consisted of all flies from Miami constrained to a monophyletic group.

When the influence of both host plant and geographical location were considered together by comparisons of single populations (for example, samples collected from population 1, flies reared from Surinam cherry from Vero Beach, Florida) there were no incidences where individuals from discrete populations formed a monophyletic group (fig. 2). Comparisons between these data and other *Anastrepha* phylogenetic studies were not possible because multiple individuals per population were not included in previous studies (McPheron *et al*., 1999; Norrbom *et al*., 1999; Smith-Caldas *et al*., 2001; Barr *et al*., 2005). One possible explanation for the paraphyly of individuals in a single population might be that high variance in oviposition-resources on spatial and temporal scales might lead to discontinuous ranges and genetic divergence among populations of fruit-infesting tephritids such as *Anastrepha* spp. (Sivinski *et al*., 2004). It is also important to note that not all flies from a given population were collected from one single fruit, but several fruits were incubated and flies that emerged from a given geographical location were labelled a population. This may mean that flies were sampled from subpopulations within a larger population. With the COI sequence variability found in one population, for example individuals from population 1 do not form a monophyletic group (figs 2 and 3), future studies of *Anastrepha* should consider including multiple individuals for a given population. Another possible explanation could be the fact that there is still gene flow between all of the populations sampled, therefore populations are not genetically distinct.

The results presented here have applicability beyond studying genetic diversity of *A. suspensa* in Florida and the Caribbean. The monophyly of all *A. suspensa* indicates the potential for use of such genetic markers for larval identification of *A. suspensa*. Our data in conjunction with morphological characters (Norrbom *et al*., 1999) and identification of other genetic markers (Schnell *et al*., 1996; Heath *et al*., 2002) that contain synapomorphic characters will undoubtedly provide valuable species-level identification tools.

Given that the inability to distinguish geographical or host preference related phylogenetic differences within *A. suspensa* may be due to incomplete lineage sorting according to COI sequence evolution, more rapidly evolving markers such as microsatellites may resolve the more recent population isolation events if they exist. We therefore plan to assess variability within *A. suspensa* at the population level by utilizing microsatellite primers designed specifically for *A. suspensa* (Fritz & Schable, 2004). Microsatellite markers have been widely used in studies involving population genetics of invasive tephritid fruit flies in Florida (Silva *et al*., 2003) and California (Meixner *et al*., 2002; Nardi *et al*., 2005), and similar approaches will be used to answer questions regarding population structure and differences, genetic drift, and gene flow.

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