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Molecules and Cells

Molecular Phylogeny of the Family Tephritidae (Insecta: Diptera): New Insight from Combined Analysis of the Mitochondrial 12S, 16S, and COII Genes

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The phylogeny of the family Tephritidae (Diptera: Tephritidae) was reconstructed from mitochondrial 12S, 16S, and COII gene fragments using 87 species, including 79 tephritid and 8 outgroup species. Minimum evolution and Bayesian trees suggested the following phylogenetic relationships: (1) A sister group relationship between *Ortalotrypeta* and *Tachinisca*, and their basal phylogenetic position within Tephritidae; (2) a sister group relationship between the tribe Acanthonevrini and Phytalmiini; (3) monophyly of *Plioreocepta*, *Taomyia* and an undescribed new genus, and their sister group relationship with the subfamily Tephritinae; (4) a possible sister group relationship of *Cephalophysa* and Adramini; and (5) reconfirmation of monophyly for Trypetini, Carpomyini, Tephritinae, and Dacinae. The combination of 12S, 16S, and COII data enabled resolution of phylogenetic relationships among the higher taxa of Tephritidae.

INTRODUCTION

The family Tephritidae is a group of primarily phytophagous flies that includes 4,448 known species arranged in 484 genera, as of December, 2003 (Norrbom, The Diptera Site - http://www.sel.barc.usda.gov/diptera/tephriti/TephClas.htm). The actual number of species is probably much higher, as many remain undescribed (Norrbom et al., 1999). This family contains some of the most significant global agricultural pests, and others are significant for their role in the biological control of weeds (White and Elson-Harris, 1992). Currently, the higher classification of the Tephritidae is in an unsatisfactory state (Foote et al., 1993; Freidberg, 1984; Han and McPheron, 1997, 1999; Hancock, 1986; Korneyev, 1999a; 1999b; Norrbom et al., 1999).

In an attempt to resolve relationships among the tephritid subfamilies and tribes, Korneyev (1999b) performed a cladistic analysis of the family based on 149 terminal taxa (either species or genera) using 112 morphological characters. Although this study yielded important phylogenetic information, the author concluded that the relationships among the subfamilies and tribes had not been satisfactorily resolved because of the prevalence of homoplasies, and the absence of genitalic character data for over half the species analyzed. In the same year, Norrbom et al. (1999) provided an updated classification of the family based upon a comprehensive review of the tephritid systematics literature. The up-to-date classification table, including 6 subfamilies and 27 tribes, is available from the abovementioned web site of the Systematic Entomology Laboratory (ARS, USDA). This information provides a starting point for working hypotheses about higher relationships that can be tested using more characters and additional taxon sampling.

As in other Tephritoidea, morphological characters have shown a mosaic character state distribution among the Tephritidae (Korneyev, 1999a), and it is extremely difficult to determine phylogenetic signals, most likely because the number of informative characters is limited (Han and Ro, 2005). Han and McPheron (1997) introduced molecular sequence data to test tephritid higher relationships. They performed a phylogenetic analysis using a 923 bp alignment of partial 16S rDNA sequence generated from a limited number of representative taxa, and found relationships that differed from those based on morphological characters, as well as several previously unsuspected relationships. McPheron and Han (1997) used the same 16S region to infer relationships among the North American *Rhagoletis* flies, resulting in a highly resolved phylogeny of the genus. At the same time, Smith and Bush (1997) published a phylogenetic analysis of North American *Rhagoletis* based on the mitochondrial COII sequences, resulting in essentially the same inferred phylogenetic tree of McPheron and Han (1997). Han and McPheron (1999) used these two studies to do a combined analysis of 16S and COII gene sequences. Han (2000) attempted to test the monophyly and subtribal classification of the tribe Trypetini based upon nearly complete 16S rDNA sequences in a 1279 bp aligned dataset, with additional sampling of representative species. The results were congruent with the previous molecular (Han and McPheron, 1997) and morphological studies (Han, 1992; 1999). More recently, Han et

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al. (2006) inferred the relationships within the subfamily Tephritinae from the 16S sequences, using 53 species representing 11 currently recognized tribes of the subfamily Tephritinae and 10 outgroup species. This study yielded an exceptionally well-resolved phylogeny that can be used to improve the higher classification as well as to provide working hypotheses for further investigation of evolutionary history.

Han and Ro (2005) reconstructed the phylogeny of the superfamily Tephritoidea from 12S, 16S, and COII gene sequences using 49 species representing 19 tephritoid and related families. The strong phylogenetic signal provided by the combination of these three mitochondrial markers allowed confident revision of the controversial classification of the superfamily. This study also showed that combined analysis using the three gene fragments is highly useful for resolving Tephritoidea phylogenetic relationships at species to family levels. In the present study, we resolve higher relationships of the family Tephritidae using these three genes. Han and McPheron's (1997) earlier study did little to improve the resolution of higher-level tephritid relationships, so we analyzed over twice the number of species, representing many additional lineages, and three times the number of molecular characters, to obtain more data to resolve the phylogenetic relationships among tribes and subfamilies.

MATERIALS AND METHODS

Taxon sampling

Currently over 4,400 described tephritid species are grouped in 6 subfamilies and 27 tribes (Norrbom, The Diptera Site). For our analysis, DNA sequence data were collected for 87 species representing major tephritid taxa and four outgroup families (Table 1). For the tephritid species, we sampled all six subfamilies and the majority of the recognized tribes and subtribes (Table 1). For the large but well-defined monophyletic subfamily Tephritinae, Han et al. (2006) inferred a reasonably resolved phylogeny based on 16S rDNA sequences of 53 species representing all 11 currently recognized tribes within the subfamily, so we sampled only five species representing the Tephritinae as a single lineage. For the other subfamilies, we were unable to obtain any samples for the small tribes Epacrocerini, Phascini, Rivelliomimini, and Xarnutini.

Eight species representing four outgroup families were selected based on the recent inferred molecular phylogeny of Tephritoidea Group-2 (Han and Ro, 2005). The resulting trees were rooted using the family Richardiidae, which appears to be the basal-most group among these families.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from freshly frozen flies following a standard protocol optimized for individual flies (Sheppard et al., 1992). Pinned or alcohol-preserved specimens were processed by the modified protocol described in Han and McPheron (1997), or since 1997, using the Qiagen DNeasy Tissue Kit following the manufacturer's protocol for animal tissues. Either a whole body or a part of a body, primarily leg(s), was used for each DNA extraction.

To design primers for amplification, the complete mitochondrial genomes available in GenBank for the following six fly species were compared: *Ceratitis capitata* (Tephritidae; NC_000857), *Cochliomyia hominivorax* (Calliphoridae; AF206826), *Drosophila melanogaster* (Drosophilidae; AF200829), *D. yakuba* (Drosophilidae; X03240), *Anopheles gambiae* (Culicidae; NC_002084), and *A. quadrimaculatus* (Culicidae; NC_000875). Table 2 provides a list of primers used for DNA amplification. A variability plot of the whole mitochondrial genome based on these sequences was also used to determine relative variability among different genes. The 12S, 16S, and COII genes contain some of the least variable areas in the genome, and thus, represent better candidates for the phylogenetic analyses of higher-level fly relationships.

The regions to be analyzed were amplified using standard PCR with the following conditions: Initial denaturation at 95°C for 3 min followed by 40 cycles at 93°C for 1 min, primer-specific annealing temperatures (45-55°C) for 1 min, and extension at 72°C for 2 min; this was followed by an final extension for 15 min at 72°C. Using the GFX PCR DNA and Gel Band Purification Kit (Amersham Bioscience), double-stranded amplification products were purified on a 2% agarose gel electrophoresis in 1X TAE buffer.

Purified products were either sequenced directly or cloned into pCR2.1-TOPO using the TOPO TA Cloning Kit Version L (Invitrogen) according to the manufacturer's protocol. Positive colonies were grown overnight in LB broth at 37°C and 150 rpm and screened for presence of the appropriate-sized insert by digesting with *Eco*R1. Plasmid DNA was isolated with the E.Z.N.A. Plasmid Miniprep Kit (Omega Bio-Tek, Inc.). Purified plasmid or PCR product was sequenced using M13-Forward, M13-Reverse, and PCR primers. COII fragments were usually sequenced directly, while the 12S and 16S fragments were cloned before sequencing. Sequencing analyses were performed using a Base Station automated DNA sequencer (MJ Research) at Bioneer Co. and ABI 377 or ABI/HITACHI 3100 sequencer at Bionics Co.

Sequence alignment and phylogenetic analysis

Raw sequences were examined and corrected using BioEdit (version 7.0.5.3, 2005; Hall, 1999), then aligned using CLUSTAL X (version 1.83, 2007; Thompson et al., 1997). No additional manual adjustment was made, but conserved blocks of the alignment were selected from the multiple-aligned sequences using GBLOCKS 0.91b (Castresana, 2002) and eliminated from the dataset. The COII sequences were aligned based on amino acid translations. There was no length variation among the included sequence.

Minimum-evolution (ME) analyses were performed in MEGA 4.0 (Kumar et al., 2007) using the Maximum Composite Likelihood model of nucleotide substitution (Yang, 1994) with different evolutionary rates among sites and lineages. The reliability of clustering patterns in trees was determined by the interior branch test (= standard error test; Rzhetsky and Nei, 1992) and bootstrap test (Felsenstein, 1985) (1,000 replications). The 12S, 16S, and COII sequences were analyzed separately as well as in various combinations to assess phylogenetic signal in terms of statistical support for interior branches and compatibility with the previously established classification (Table 1).

Bayesian inferences (BI) were conducted using MrBayes v3.0b4 (Huelsenbeck and Ronquist, 2001) by Markov chain Monte Carlo (MCMC) sampling for one million generations, with tree sampling every 100 generations and a burn-in of 500 trees. ModelTest 3.06 (Posada and Crandall, 1998) used in conjunction with PAUP 4b10 (Swofford, 2002), determined that the GTR + I + G model (Gu et al., 1995; Lanave et al., 1994) provided the best fit to the data according to both the likelihood-ratio test and the AIC criterion. Using MrBayes, we constructed majority-rule consensus trees for 9,500 sampled trees. The analyses were run twice using different random starting trees to evaluate the congruence of the likelihood values and posterior clade probabilities (Huelsenbeck et al., 2002). Three gene regions were analyzed as partitioned datasets or combined datasets.

Table 1. Collection and voucher information for flies used in this study. Status of the voucher specimens and specimen depositories with voucher numbers and GenBank accession numbers are indicated in parentheses. See Norrbom et al. (1999) and Korneyev (1999a; 1999b) for classification of the Tephritidae, http://www.sel.barc.usda.gov/diptera/tephriti/TephClas.htm for further information. Sequence data newly generated in this study are represented by the GenBank Accession Nos. EU926784-EU926954.

OUTGROUP (Tephritoidea Group-2, Han and Ro (2005))

Family Richardiidae

1. *Automola atomaria* Wiedemann. BRAZIL: Rondonia 62 km S. Ariquemes, Faz. Rancho Grande, 11-22.XI.1992, N. M. Schiff (5 individuals from same collecting lot; YSUW92111003; 12S-**AY573068**, 16S-**AY573109**, COII-**AY573143**).
2. *Odontomera nitens* Schiner. GUATEMALA: Escuintala: Palin area, McPhail trap,1992-1993, J. Lopez (both wings glued on rectangular card; YSUW94082629; 12S-**AY573087**, 16S-**AY573123**, COII-**AY573162**).

Family Ulidiidae

3. *Delphinia picta* (Fabricius). USA: Illinois, Pope Co., Dixon Sprs Ag Cntr, malaise trap, 24.V.1987, N.M. Schiff (both wings glued on rectangular card; YSUW92121509; 12S-**AY573075** 16S-**AF177121**; COII-**AY573150**).
4. *Euxesta notata* Wiedemann. USA: PA: Centre Co., W. of Spring Creek Hatchery, swept from dead fish,16.VI.1992, H.-Y. Han (1 male from same collecting lot; YSUW92121506; 12S-**AY573080**; 16S-**AY573118**; COII-**AY573155**).

Family Platystomatidae

5. *Platystoma seminationis* (Fabricius). SWITZERLAND: ZH, 410 m, Glattfelden-Bhf, 15.V.1993, B. Merz (both wings glued on rectangular card; YSUW93092303; 12S-**AY573093**; 16S-**AY573128**; COII-**AY573168**).
6. *Rivellia alini* Enderlein. KOREA: Gangwon-do, Samcheog-si, Hajang-myeon, Mt. Jungbongsan, 23.VII.1997, H.-Y. Han et al. (both wings glued on rectangular card; YSUW00032307; 12S-**AY573099**; 16S-**AY573132**; COII-**AY573174**).

Family Pyrgotidae

7. *Adapsilia verrucifer* Hendel (= *Adapsilia cornugaster* Kim & Han). KOREA: Gangwon-do, Wonju-si, Guyrae-myeon, Guyrae-ri, black & mercury vapor light trap, 5.VII.1996, H.-Y. Han & H.-W. Byun (both wings glued on rectangular card; YSUW00032303; 12S-**AY573065**, 16S-**AY573108**, COII-**AY573140**).
8. *Pyrgota undata* Wiedemann. USA: PA: Centre Co., W. of Spring Creek Hatchery, 30.V.1990, H.-Y. Han (both wings glued on rectangular card; YSUW92091101; 12S-**AY573097**, 16S-**AY123352**, COII-**AY573172**).

INGROUPS (Tephritidae)

Subfamily Tachiniscinae

Tribe Ortalotrypetini

9. *Ortalotrypeta isshikii* Matsumura. JAPAN: Yunosawa Pass, Enzan City, Yamanashi Pref. Honshu Japan, 17-VII-2001, M. Sueyoshi (female with 2 right legs detached; YSUW05021604; 12S-**EU926877**, 16S-**EU926935**, COII-**EU926812**).

Tribe Tachiniscini

10. *Tachinisca cyaneiventris* Kertész. BOLIVIA: La Pax: 50 km N. Caranavi, 15°9.502'S 67°6.57'W, 1100 m, 17.iv.2004 P.H. Kerr (frozen tissue collection, CSCA, 2004PHK223-01; 12S-**EU926907**, 16S-**EU926949**, COII-**EU926838**).

Subfamily Blepharoneurinae

11. *Blepharoneura femoralis* Wulp. MEXICO: Morelos: jct. Rt. 95 (libre), Km 65, jct. road to Huitzilac, ex fruit of *Cyclanthera* sp., col. 27.IX.1991, em. IX.1992 (both wings glued on rectangular card, PSU94020309; 12S-**EU926854**, 16S-**U39366**.
12. *Blepharoneura manchesteri* Condon & Norrbom. VENEZUELA: Miranda, Guatopo National Park, ex seeds of Gurania spinulosa, col. & em. V-VI.1992, M. Condon (both wings glued on rectangular card, PSU92121508; 12S-**AY573069**, 16S-**AY573110**, COII-**AY573144**).

Subfamily Phytalmiinae

Tribe Acanthonevrini

13. *Acanthonevra pteropleuralis* Hendel. KOREA: Gangwon-do, Inje-gun, Misan-1-ri, near Gaein Mineral Spring, 2.VIII.1996, H.-Y. Han & H.-W. Byun, female (both wings glued on rectangular card; YSUW97051306; 12S-**EU926846**, 16S-**EU926917**, COII-**EU926784**).
14. *Acanthonevra* sp. THAILAND: S. Khao Sok Nat. Park, Rt. 401, 22.X.1993, F. Kaplan & A. Freidberg (both wings glued on rectangular card; PSU94072005; 12S-**AY573064**, 16S-**AF177122**, COII-**AY573139**).
15. *Orienticaelum femoratum* Shiraki. KOREA: Gangwon-do, Seoseok-myeon, Sangok-ri, on rt. 56, 8.VIII.1997, H.-Y. Han et al., male (both wings glued on rectangular card; YSUW02121214; 12S-**EU926875**, 16S-**EU926934**, COII-**EU926810**).

Tribe Epacrocerini - not sampled

Tribe Phascini - not sampled

Tribe Phytalmiini

16. *Phytalmia alcicornis* (Sounders). NEW GUINEA, 2001, G. Dodson, male (head & wings glued on rectangular card; YSUW04010629; 12S-**EU926887**, 16S-**EU926939**, COII-**EU926819**).
17. *Phytalmia cervicornis* Gerstaecker. NEW GUINEA, 2001, G. Dodson, male (head & wings glued on rectangular card; YSUW04010630; 12S-**EU926888**, 16S-**EU926940**, COII-**EU926820**).

Phytalmiinae *Incertae Sedis*

18. *Matsumurania sapporensis* (Matsumura). JAPAN: Ogawa, Kitaibaraki City, Ibaraki Prefecture, Malaise trap (Orange 5-5), 2002, M. Sueyoshi (female with 3 right legs detached; FFPRI-Orange-5-5; 12S-**EU926872**, 16S-**EU926932**, COII-**EU926807**).

Subfamily Dacinae

Tribe Ceratitidini

19. *Capparimyia savastani* (Martelli). TUNISIA: Mides, IX.1994, male (DNA extract only; PSU95011908; 12S-**EU926855**, 16S-**EU926923**, COII-**EU926792**).
20. *Ceratitis capitata* (Wiedemann). Sequences from GenBank (**NC_000857**).

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21. *Ceratitis rosa* Karsch. KENYA: Dangoutti, Mutuini, 3.IX.1994, ex *Eriobotrya japonica*, male (DNA extract only; PSU95011909; 12S-**EU926859**, 16S-**EU926927**, COII-**EU926795**).
22. *Proanoplomus japonicus* Shiraki. JAPAN: Ogawa, Kitaibaraki City, Ibaraki Prefecture, Malaise trap, 2002, M. Sueyoshi (male with 3 right legs detached; FFPRI-Orange-5-5; 12S-**EU926890**, 16S-**EU926942**, COII-**EU926823**).
23. *Trirhithrum coffeae* Bezzi. KENYA: Kabete University, 1900 m, 4.III.1993, B. Merz (both wings glued on rectangular card; YSUW95022808; 12S-**EU926911**, 16S-**EU926951**, COII-**EU926842**).

Tribe Dacini

24. *Bactrocera cucurbitae* (Coquillett). USA: HAW: Hilo, USDA and Dolphin Bay, 1991, male (2 frozen specimens in same collecting lot; YSUW95011901; 12S-**EU926852**, 16S-**EU926921**, COII-**EU926790**).
25. *Bactrocera dorsalis* (Hendel). USA: OAHU: Waimanolo, ME (coffee), 1991, male (2 frozen specimens in same collecting lot; YSUW95011901; 12S-**EU926853**, 16S-**EU926922**, COII-**EU926791**).
26. *Bactrocera oleae* (Rossi). Sequences from GenBank (**NC_005333**).
27. *Dacus vertebratus* Bezzi. MALAWI: Chirombo Bay, Cuelure trap, 20-27.II.1993, N. Bowers (left wing glued on rectangular card; PPSU94040808; 12S-**EU926861**, 16S-**U39384**, COII-**EU926797**).

Tribe Gastrozonini

28. *Acrotaeniostola sexvittata* Hendel. KOREA: Gangwon-do, Inje-gun, Mt. Jeombongsan, Gangseon-ri, 7.VIII.1997, H.-Y. Han et al., male (both wings glued on rectangular card; YSUW98071108; 12S-**EU926848**, 16S-**EU926919**, COII-**EU926786**).
29. *Paragastrozona japonica* (Myiake). JAPAN: Hokkaido: Sapporo, Jozankei, 42 58'N 141 40'E, 350 m, 1-10.VIII.1989, K. Maeto & M. J. Sharkey (both wings glued on rectangular card, PSU94040803, 16S-**U39385**); KOREA: Gangwon-do, Pyeonchang-gun, N. of Rt. 6, south valley of Mt. Taegisan, 3.VII.1996, H.-Y. Han et al., male (both wings glued on rectangular card; YSUW97051308; 12S-**EU926878**, COII-**EU926815**).

Subfamily Trypetinae

Tribe Acidoxanthini – not sampled

Tribe Adramini

30. *Adrama apicalis* Shiraki. THAILAND: Khao Lak National Park, ex undet. large seeds picked up from ground, 20.X.1993, A. Freidberg (both wings glued on rectangular card, PSU94040801, 12S-**EU926849**, 16S-**U39387**, COII-**EU926787**).
31. *Euphranta canadensis* (Loew). USA: Wyoming, 17-18.VII.1979, S.H. Berlocher and G.J. Steck, male (both wings glued on rectangular card, PSU94020301, 12S-**EU926865**, 16S-**AF177126**, COII-**EU926801**).
32. *Euphranta oshimensis* (Shiraki) KOREA: Gangwon-do, Wonju-si, Heungeop-myeon, Gwirae-ri, Cheoneunsa Temple to Mt. Sipjabong, 19.VIII.1997, HY Han et al., female (both wings glued on rectangular card; YSUW02121215; 12S-**EU926866**, 16S-**EU926930**, COII-**EU926802**).

Tribe Carpomyini

Subtribe Carpomyina

33. *Carpomya incompleta* (Becker). ISRAEL: Ein Gedi, 300 m, 10.IV.1992, B. Merz (left wing glued on rectangular card; YSUW95022810; 12S-**EU926856**, 16S-**EU926924**).
34. *Carpomya schineri* (Loew). HELV. VS 850m Betten-Telstat. 19.VII.1991, B. Merz (both wings glued on rectangular card; YSUW95022805; 12S-**EU926857**, 16S-**EU926925**, COII-**EU926793**).
35. *Cryptodacus tau* (Foote). SWITZERLAND: VS, 850 m, Betten-Telstat, 19.VII.1991, B. Merz; PSU94040802; 16S-**U39414**).
36. *Goniglossum* sp. ISRAEL: Alma, ex fruit of *Bryonta cretica*, col. 10.XI.1992, pup. 11.VI.1992, em. 6.VII.1992, A. Freidberg (both wings glued on rectangular card; PSU94072001; 12S-**EU926867**, 16S-**U39388**, COII-**EU926803**).
37. *Haywardina cuculi* (Hendel). ARGENTINA: Tucuman, Burruyacu, Taruca Pampa, Finca San Augustine, ex fruit of *Solanum trichoneuron*, col. 18.V.1991, A. L. Norrbom (both wings glued on rectangular card; PSU94022801, 12S-**EU926869**, 16S-**U39416**).
38. *Rhagoletotrypeta pastranai* Aczél. BRAZIL: Santa Catarina, Cocador, ex Celtis iguanae, col. 16.III.1987 (both wings glued on rectangular card; PSU94022802, 12S-**EU926903**, 16S-**U39417**).
39. *Zonosemata electa* (Say). USA: Pennsylvania: Centre Co., Penn State campus, ex *Juglans cinerea* fruit, col. IX.1988, em. 1989, B. A. McPheron (1 male and 1 female from same collecting lot; PSU94020303; 12S-**EU926916**, 16S-**U39415**, COII-**EU926845**).
40. *Rhagoletis berberidis* Jermy. Czech or Slovak (5 flies from same collecting lot; PSU94072010; 12S-**EU926894**, 16S-**EU926945**, COII-**EU926826**).
41. *Rhagoletis cerasi* (Linnaeus). HUNGARY: ex fruit of *Prunus avium* (1 male and 1 female from same collecting lot; PSU94020307; 12S-**EU926895**, 16S-**U39389**, COII-**EU926827**).
42. *Rhagoletis magniterebra* (Rohdendorf). KYRG: Kyrghyz Alarau, Bo-Om revin, 1900m, 23.6. VI. 1996, Kameneva (both wings glued on rectangular card; YSUW02121206; 12S-**EU926899**, 16S-**EU926946**, COII-**EU926831**).
43. *Rhagoletis cingulata* (Loew). USA: Illinois: Champaign Co., Lake of the Woods, ex *Prunus serotina* fruit, 1986, B.A. McPheron (1 male and 1 female from same collecting lot; PSU93032902; 12S-**EU926896**, 16S-**U39427**, COII-**EU926828**).
44. *Rhagoletis fausta* (Osten Sacken). USA: Wisconsin: Door Co., 1984, J. Feder (1 male and 1 female from same collecting lot; PSU93032908; 12S-**EU926898**, 16S-**U39433**, COII-**EU926830**).
45. *Rhagoletis cornivora* Bush. (USA: Pennsylvania: Centre Co., Houserville, ex *Cornus amomum* fruit, 1989, B.A. McPheron (1 male and 1 female from same collecting lot; PSU93032904; 12S-**EU926897**, 16S-**U39420**, COII-**EU926829**).
46. *Rhagoletis pomonella* (Walsh). (USA: Pennsylvania: Centre Co., Penn State campus, ex *Crataegus mollis* fruit, col. IX.1989, em. VIII.1990, B.A. McPheron; PSU93032912; 12S-**EU926900**, 16S-**AF177127**, COII-**EU926832**).
47. *Rhagoletis striatella* Wulp. (USA: Pennsylvania: Centre Co., Pleasant Gap, ex *Physalis subglabrata* fruit, col. 3.IX.1989, em 15–22.VI.1990, H.Y. Han (1 male and 1 female from same collecting lot; YSUW92062312; 12S-**EU926901**, 16S-**U39418**, COII-**EU926833**).
48. *Rhagoletis suavis* (Loew). USA: Pennsylvania: Centre Co., Penn State campus, ex *Juglans cinerea* fruit, col. IX.1988, em. 1989, B. A. McPheron (1 male and 1 female from same collecting lot; PSU93032914; 12S-**EU926902**, 16S-**U39419**, COII-**EU926834**).

(continued)

Subtribe Notommatina

49. *Malica caraganae* Richter. KYRG: Kyrghyz Alarau, Bo-Om revin, 1900m, 23.6. VI. 1996, Kameneva (both wings glued on rectangular card; YSUW02121207; 12S-**EU926871**, 16S-**EU926931**, COII-**EU926806**).

Subtribe Paraterelliina

50. *Oedicarina latifrons* (Wulp). MEXICO: Mexico: Rt. 890, km 9, ex fruit of *Solanum brachycarpum*, col. 1991, em. IX.1992, A. L. Norrbom (both wings glued on rectangular card; PSU94020308; 12S-**EU926874**, 16S-**U39413**, COII-**EU926809**).

Tribe Nitrariomyiini

51. *Nitrariomyia lukjanovitshi* Rohdendorf. ISRAEL: Hazera, puparium ex *Nitraria retusa* berry, V.1998, A.Freidberg (9 pupae in freezer; YSUW98071001; 12S-**EU926873**, 16S-**EU926933**, COII-**EU926808**).

Tribe Rivelliomimini – not sampled

Tribe Toxotrypanini

52. *Anastrepha serpentina* (Wiedemann). VENEZUELA: Maraca, 1988, G. Steck (1 male and 1 female from same collecting lot; YSUW04003; 12S-**AY573066**, 16S-**AF177125**, COII-**AY573141**).
53. *Hexachaeta amabilis* (Loew). GUATEMALA: Escuintala: Palin area, McPhail trap, 1992-1993, J. Lopez (both wings glued on rectangular card; PSU94062108; 16S-**U39382**)
54. *Toxotrypana curvicauda* Gerstaecker. USA: Florida, 1990, J. Sivinski (1 male and 1 female from same collecting lot; PSU92062915; 12S-**EU926910**, 16S-**U39381**, COII-**EU926841**).

Tribe Trypetini

Subtribe Chetostomatina

55. *Chetostoma curvinerve* Rondani. ISRAEL: Mt. Meron 1200 m, 16.IV.1992, A. Freidberg (both wings glued on rectangular card; PSU94072002; 16S-**AF177131**); ISRAEL: Har Meron 1100 m, 20.VIII.1990, A. Freidberg (both wings glued on rectangular card; YSUW06011027; 12S-**EU926860**, COII-**EU926796**).
56. *Parastenopa elegans*? (Blanchard). PANAMA: Altos de Pacora, 3 Mar 2002, C.A. Korytkowski, McPhail traps, female (both wings glued on rectangular card; YSUW02121211; 12S-**EU926882**, 16S-**EU926938**).
57. *Parastenopa limata* (Coquillette). USA: Pennsylvania: Centre Co., Penn. State Univ. campus, west of Frear Bldg. ex fruit of *Ilex verticilata*, col. 28.IX.1991, H.-Y. Han (male and female from the same collecting lot; YSUW92112302; 12S-**EU926880**, 16S-**AF177128**, COII-**EU926814**).
58. *Parastenopa* n.sp.-A. PANAMA: Altos de Pacora, I-V.2002, C.A. Korytkowski, McPhail trap, male (both wings glued on rectangular card; YSUW04010614; 12S-**EU926879**, 16S-**EU926937**, COII-**EU926813**).
59. *Parastenopa* n.sp.-B. PANAMA: Altos de Pacora, 2001, C.A. Korytkowski, McPhail trap, female (same specimen with right 3 legs detached; USNM00216079; 12S-**EU926881**, 16S-**EU926936**).
60. *Prochetostoma* n.sp. JAPAN, 27.VI.2002, K. Takachi, female (both wings glued on rectangular card; YSUW02121208; 12S-**EU926891**, 16S-**EU926941**, COII-**EU926822**).
61. *Sinacidia esakii* (Ito). KOREA: Gangwon-do, Jeongseon-gun, Mt. Mindungsan, 19.VII.2005, Han et al., male (same specimen with right mid & hind legs detached; YSUW06010904; 12S-**EU926904**, 16S-**EU926947**, COII-**EU926835**).
62. *Sinacidia flexuosa* (Zia). KOREA: Gangwon-do, Wonju-si, Heungeop-myeon, Yonsei Univ. Campus, 3.IV.2006, H.-W. Byun, female (same specimen with right mid & hind legs detached; YSUW06040602; 12S-**EU926905**, 16S-**EU926948**, COII-**EU926836**).

Subtribe Trypetina

63. *Acidiella* sp. KOREA: Gangwon-do, Wonju-si, Heungeop-myeon, Hoechon, 8.VI.1996, H.Y. Han & H.W. Byun, male (same specimen with 1 leg detached; YSUW03012206; 12S-**EU926847**, 16S-**EU926918**, COII-**EU926785**).
64. *Anastrephoides matsumurai* Shiraki. KOREA: Gangwon-do, Wonju-si, Heungeop-myeon, Hoechon, 1.VII.1996, H.Y. Han & H.W. Byun, female (left wing glued on rectangular card, right wing slide mounted; YSUW98071106; 12S-**EU926851**, 16S-**EU010373**, COII-**EU926789**).
65. *Dryadodacryma continuum* Ito, JAPAN: Ogawa, Kitaibaraki City, Ibaraki Prefecture, Malaise trap (Blue 5-2), 2002, M. Sueyoshi, female (same specimen with 3 right legs detached; FFPRI-Blue-5-2; 12S-**EU926862**, 16S-**EU926928**, COII-**EU926798**).
66. *Euleia fratria* (Loew). USA: PA: Centre Co., along Spring Creek, west of fish hatchery, reared from leaf mine of *Angelica atropurpurea*, col. 23.VIII.1989, em 6-12.IX.1989, H.-Y. Han (1 male and 1 female from same collecting lot; YSUW92022702; 12S-**EU926864**, 16S-**AF177136**, COII-**EU926800**).
67. *Hemileophila sibirica* (Portschinsky). KOREA: Gangwon-do, Wonju-si, Heungeop-myeon, Hoechon, 8.VI.1996, H.Y. Han & H.W. Byun, male (both wings glued on rectangular card; YSUW97051301; 12S- **EU926870**, 16S-**AF177142**, COII- **EU926805**).
68. *Itosigo bellus* Ito. KOREA: Gangwon-do, Inje-gun, Mt. Jeombongsan, 18.VIII.1996, H.-Y. Han & H.-W. Byun, male (both wings glued on rectangular card; YSUW97051311; 12S-**AY573081**, 16S-**AF177133**, COII-**AY573156**).
69. *Philophylla caesio* (Harris). SWITZERLAND: SG 400m, Attenrhein, 4.VIII.1987, B. Merz, female (both wings glued on rectangular card; YSUW94022804; 12S- **EU926883**, 16S-**EU010375**).
70. *Philophylla fossata* (Fabricius). KOREA: Gangwon-do, Pyeongchang-gun, Yongpyeong-myeon, Mt. Gyaebangsan, south valley, 10.VIII.1996, H.Y. Han & H.W. Byun, male (same specimen with 1 leg detached; YSUW98071305; 12S-**EU926884**, 16S-**EU010377**, COII-**EU926816**).
71. *Philophylla millei* Han & Norrbom. NEW CALEDONIA: Sarramea Col d'Amieu, 8.III.2006, Malaise trap, C. Mille, male (holotype with left mid & hind legs detached; MNHNP (YSUW06040601); 12S- **EU926885**, 16S-**EU010378**, COII- **EU926817**).
72. *Philophylla pulla* (Ito). KOREA: Gyeonsangnam-do, Geoje-si, Mt. Nojasan, 4-5.VI.1997, D.S. Ku, female (both wings glued on rectangular card; YSUW98071104; 12S-**EU926886**, 16S-**EU010374**, COII-**EU926818**).
73. *Vidalia armifrons* (Portschinsky). JAPAN: Ogawa, Kitaibaraki City, Ibaraki Prefecture, Malaise trap, 2002, M. Sueyoshi, female (same specimen with 3 right legs detached; FFPRI-Red-4; 12S-**EU926914**, 16S-**EU926953**, COII-**EU926844**).
74. *Vidalia? rohdendorfi* Richter. JAPAN: Ogawa, Kitaibaraki City, Ibaraki Prefecture, Malaise trap, 2002, M. Sueyoshi, female (same specimen with 3 right legs detached; FFPRI-Brown-11-2A; 12S-**EU926915**, 16S-**EU926954**).

(continued)

Tribe Xarnutini - not sampled

Tribe Zaceratini

75. *Plioreocepta poeciloptera* (Schrank). SWITZERLAND: Wallis ohne Simplonsüdseite 520 m, 19.V.1989, B. Merz (both wings glued on rectangular card; PSU94022805; 12S-**EU926889**, 16S-**U39377**, COII-**EU926821**).

Trypetinae *Incertae Sedis*

76. *Alujamyia bella* Norrbom. MEXICO: Veracruz: Apazapan Huerta de la Sra. Lticia Lagunes. 16.II.2000, M. Aluja, McPhail trap with protain & borax in *Mangifera indica* tree, male (same specimen with right fore & mid legs detached; USNM00055185; 12S-**EU926850**, 16S-**EU926920**, COII-**EU926788**).
77. *Cephalophysa terebratula* (Portschinsky). KOREA: Gangwon-do, Hongcheon-gun, Naemyeon, n. valley of Mt. Hoeryeongbong, 26.IV.1998, H.-Y. Han et al., female (same specimen with right hind leg detached; YSUW03012205; 12S-**EU926858**, 16S-**EU926926**, COII-**EU926794**).
78. *Esacidia kuwayamai* Ito. KOREA: Gangwon-do, Inje-gun, Mt. Jeombongsan, Gangseon-ri, 23.VIII.1997, H.-Y. Han et al., female (same specimen with left hind leg detached; YSUW98071301; 12S- **EU926863**, 16S- **EU926929**, COII- **EU926799**).
79. *Pseudophorellia acrostichalis* Norrbom. BOLIVIA: La Paz: Nor Yungas, above Coroico, Cerro Uchumachi, summit, 16.IV.2001, A.L. Norrbom & G.A. Kung, Holotype male (same specimen with right mid & hind legs detached; USNM00056108; 12S-**EU926892**, 16S-**EU926943**, COII-**EU926824**).
80. *Pseudophorellia semilunata* Norrbom. PANAMA: Parque Nacional Chagres, Altos de Pacora, fogging canopy, 25.V.2002, Korytkowski, Holotype female (same specimen with right mid & hind legs detached; USNM00215497; 12S- **EU926893**, 16S-**EU926944**, COII-**EU926825**).
81. *Taomyia marshalli* Bezzi. KENYA, Western Prov., Kakamega Forest, 29.I.2002, R.S. Copeland, 001413N, 345187E, 1550m, male (both wings glued on rectangular card; YSUW06091609; 12S-**EU926908**, 16S-**EU926950**, COII-**EU926839**).
82. New Genus and Species. KOREA: Chungcheongnam-do, Seosan-si, Daesan-eub, Daesan-1-ri, Mangilsa Temple, 2006.V.20, Lee Heung-Sik, N36 56 29.8 E126 26 85.1, 184 m, male (same specimens with abdomen kept in genitalia vial; YSUW06091606; 12S-**EU926913**, 16S-**EU926952**, COII-**EU926843**).

Subfamily Tephritinae (10 tribes currently recognized)

83. *Gymnocarena mexicana* (Aczél). MEXICO: Michoacan, 1-2 km north of Angangueo, ex flower of *Dahlia imperialis*, 4.X.1991, A.L. Norrbom (both wings glued on rectangular card; PSU92112311; 12S-**EU926868**, 16S-**U39378**, COII-**EU926804**).
84. *Orotava hamula* (de Meijere). KOREA: Gangwon-do, Wonju-si, Heungeop-myeon, Gwirae-ri, Cheoneunsa Temple to Mt. Sipjabong, 19.VIII.1997, HY Han et al., female (both wings glued on rectangular card; YSUW02121216; 12S-**EU926876**, 16S-**DQ471408**, COII-**EU926811**).
85. *Sphaeniscus atilius* (Walker). KOREA: Jeju-do, Jeolmul Recreation Forest, 25.VI.2003, Han et al., male (both wings glued on rectangular card; YSUW04010604; 12S-**EU926906**, 16S-**DQ471397**, COII-**EU926837**).
86. *Tephritis signatipennis* Foote. USA: Utah: Grand Co., La Sal Mt. Warner Lake3, 7.IX.1992, A.L. Norrbom (3 males and 3 females from same collecting lot; PSU92112313; 12S-**EU926909**, 16S-**AF177124**, COII-**EU926840**).
87. *Trupanea amoena* (Frauenfeld). ISRAEL: Nahel Qumran, 10.IV.1992, Merz & Freidberg (both wings glued on rectangular card; YSUW94082635; 12S-**EU926912**, 16S-**DQ471412**).

We analyzed a simple three-gene partition set as well as a five partition set with COII partitioned at 1st, 2nd, and 3rd codon positions. For the combined analyses, we included either the complete COII sequences or the 1st and 2nd codon positions only.

Sequence availability and voucher specimens

Sequence data newly generated in this study have been deposited in GenBank (Accession Nos. EU926784 - EU926954; see also Table 1). Some previously submitted sequences (U39366, U39381-U39382, U39384-U39385, U39387-U39389, U39413-U39420, U39427, and U39433) were also updated as a result of the present study. Approximately 300 bases have been appended to the 3′ end of each of these sequences. The aligned data set is reproducible using the method described above, but is also available as Nexus or MEGA files upon request.

The species names, collection and voucher data, and GenBank accession numbers are provided in Table 1. Except for a few taxa whose sequences were available from Genbank, flies were either collected by us or donated by other dipterists. As shown in Table 1, vouchers for this study were either additional specimens with same collection data or body parts (mostly wings) from the specimens sequenced. The followings are the acronyms for the institutions where the voucher specimens are deposited (see also Table 1): CDFA, Collection of Arthropods, Analysis and Identification Unit, California Department of Food and Agriculture, 1220 N. St., Rm 340, Sacramento, CA 95814, USA; FFPRI, Forest Zoology Group, Kyushu Research Center, Forestry and Forest Products Research Institute, 4-11-16 Kurokami, Kumamoto 860-0861, Japan. MNHNP, Museum National d'Histoire Naturelle, National Collection of Insects, 45, rue Buffon, Paris 75005, France; PSU, Department of Entomology, Pennsylvania State University, University Park, Pennsylvania, USA; USNM, National Museum of Natural History, Washington, D.C. 20013-7012, USA; YSUW, Division of Biological Science and Technology, College of Science and Technology, Yonsei University, Gangwon-do 220-710, Korea.

RESULTS

Characteristics of the three gene fragments

For the 12S data set, the number of aligned sites was 775 bp, but 664 bp were used for analyses after excluding sites of ambiguous alignment identified using the Gblocks analysis (default setting except for allowed gap = with half). For this gap option, only the positions where 50% or more of the sequences have a gap are treated as a gap position which is eliminated from the alignment. However, positions with a gap in less than 50% of the sequences can be selected in the final alignment if they are within an appropriate block. Among the selected 664 sites, 377 were variable and 276 were parsimony-informative. The uncorrected sequence divergence among taxa for 12S rDNA ranged from 1.4% to 16.7% with an average of 9.5%. The average T:C:A:G ratio was 40:9:36:15 with a narrow standard error around the mean, but the base composition varied substantially

Table 2. Oligonucleotides used for DNA amplification and sequencing

Target gene	Primer name	Alias	Sequence
12S	SR-N-14880	A12DD	5′-TTTATATGTAAATTTTTGTGTG-3′
	SR-N-14923	A12X	5′-TTAAAGTTTTATTTTGGCTT-3′
	SR-N-14588	A12C	5′-CTAGGATTAGATACCCTATTAT-3′
	SR-J-14382	S12F	5′-CTACACCTTGATCTGATATA-3′
	SR-J-14176	S12A	5′-CATTCTAGATACACTTTCCAGT-3′
16S	TV-N-14114	A16DD	5′-GTAAAGCATTTCATTTACATTG-3′
	LR-N-13769	A16G	5′-GAAATGAAATGTTATTCGTT-3′
	LR-J-13677	S16B	5′-AGCTTATCCCATAAAATATT-3′
	LR-N-13395	A16S	5′-CCTGTTTAACAAAAACATGTC-3′
	LR-J-13415	S16T	5′-GACATGTTTTTGTTAAACAGG-3′
	LR-J-13323	S16A	5′-ACTAATGATTATGCTACCTT-3′
	LR-J-13996	S16H	5′-TTTATAGGGTCTTCTCGTC-3′
	LR-J-12883	S16R	5′-CACCGGTTTGAACTCAGATC-3′
COII	TL2-J-3045	JCO2A	5′-GATTAGTGCAATGGATTTAAGC-3′
	TL2-J-3032	JCO2C	5′-CTAATATGGCAGATTAGTGC-3′
	TK-N-3768	NCO2B	5′-ACTTGCTTTCAGTCATCTAATG-3′
	TK-N-3814	NCO2D	5′-TTAGAAGTAAGTGCTAATTTAC-3′

The standardized protocol by Simon et al. (1994) was followed for naming primers. *Drosophila yakuba* (Clary and Wolstenholme, 1985) was used as a reference sequence. Aliases are primer codes used in HYH's lab.

at different regions within the sequences.

For the 16S data set, the number of aligned sites was 1389 bp, but 1118 bp were used for analyses after excluding sites of ambiguous alignment identified using the Gblocks analysis as described above. Among these 1118 sites, 658 were variable and 498 were parsimony-informative. The uncorrected sequence divergence among taxa for 16S sequences ranged from 1.4% to 17.9% with an average of 8.7%. The average T:C:A:G ratio was 44:6:37:13 with a narrow standard error around the mean, but the base composition also varied substantially in different regions within the sequences.

Alignment of COII sequences was straightforward with no gaps, as expected. Among 669 aligned sites, 393 were variable and 322 were parsimony-informative. The uncorrected sequence divergence among taxa for COII sequences ranged from 5.2% to 26.1% with an average of 15.9%, but these values varied at different codon positions (1^{st}-10.8%; 2^{nd}-2.7%; 3^{rd}-34.3%). The average T:C:A:G ratio was 37:16:34:13 with a narrow standard error around the mean, but the base composition varied substantially at different codon positions (1^{st}-26:19:32:23; 2^{nd}-40:20:26:13; 3^{rd}-44:11:43:2).

After the exclusion of sites of ambiguous alignment, the complete data set of 12S and 16S rDNAs and COII consisted of 2451 bp, of which 1407 were variable and 874 were parsimony-informative. The uncorrected sequence divergence among taxa for the complete data set ranged from 2.8% to 19.9% with an average of 11.2%. When the 3^{rd} codon positions of COII sequences were eliminated, the data set consisted of 2228 bp, of which 1196 were variable and 773 were parsimony-informative. The uncorrected sequence divergence among taxa for the complete data set ranged from 1.6% to 16.6% with an average of 8.7%.

Phylogenetic analyses

The phylogenetic signal of the 12S, 16S, COII, and combined datasets was assessed by comparing the resulting inferred phylogenetic trees with well-established portions of the current classification for compatibility and statistical support for the interior branches.

In the ME trees (Figs. 1 and 2), P values from the interior branch test (Pc) tended to be higher than bootstrap P values (Pb). As in previous molecular studies based on 12S, 16S, and COII genes (Han, 2000; Han and McPheron, 1997; 1999; Han and Ro, 2002; 2005; Han et al., 2006), we interpret branches supported both by at least 95% of Pc and 70% of Pb as highly likely to represent the true phylogeny. In the ME analyses of the individual genes, we recognized 17 nodes with high statistical support in the 12S tree, 29 nodes in the 16S tree, and 12 nodes in the COII tree (Fig. 1). Considering the length differences in these three fragments, the 12S and 16S fragments appear to be similarly informative, but the COII fragment of similar length to the 12S was less informative for this analysis. Therefore, we eliminated the most variable third codon positions in COII (34.3% sequence divergence) in the combined analyses as these sites are likely to be saturated. The average sequence divergences of 12S, 16S, and COII (excluding 3^{rd} codon positions) were 9.5%, 8.7%, and 6.7% respectively.

Our prior study (Han and Ro, 2005) indicated that phylogenetic signal was enhanced when two or more gene fragments were combined. In the ME analyses, no conflicting relationships were statistically supported (both $Pc \geq 95$ and $Pb \geq 70$) by different datasets or combination of datasets. In any case, adding the COII fragment without the third codon position was more informative (Fig. 2).

We analyzed variously partitioned datasets for Bayesian inference (BI) (see "Materials and Methods"), and the non-partitioned dataset excluding the third codon positions of the COII fragment yielded the best-resolved tree (Fig. 3), with 42 nodes having over 95% posterior probability (Pp). Huelsenbeck and Rannala (2004) indicated that if the investigator's confidence in a particular group is 95%, then ideally the grouping of taxa would be incorrect 5% of the time, on repeated sampling. Based on the simulations using various evolutionary models, they concluded that the Bayesian method is more sensitive to underspecification of the evolutionary model than to overspecification. Under the assumption that the ModelTest software recovered a sufficiently specific model for our dataset, we interpreted over 95% of Pp to be statistically significant.

When compared with well-established portions of the current classification, the BI tree (Fig. 3) seemed slightly more congruent than the ME tree (Fig. 2B), especially in the basal phylogenetic branches. For example, the family Tephritidae and the subfamily Phytalmiinae were supported as monophyletic groups only in the BI tree. Our discussion, therefore, is based mostly on the BI tree (Fig. 3).

DISCUSSION

Monophyly of Tephritidae

Only the BI tree recovered all species representing the family Tephritidae as a monophyletic group, although without significant statistical support (Fig. 3-Node 1). However, our earlier study using the same mitochondrial gene regions based on five species of the Tephritidae and 28 species representing all other recognized families of the Tephritoidea (Han and Ro, 2005) strongly supported the monophyly of the Tephritidae. We are unsure whether support for the monophyly of this family in the present analysis is obscured by the introduction of a number of

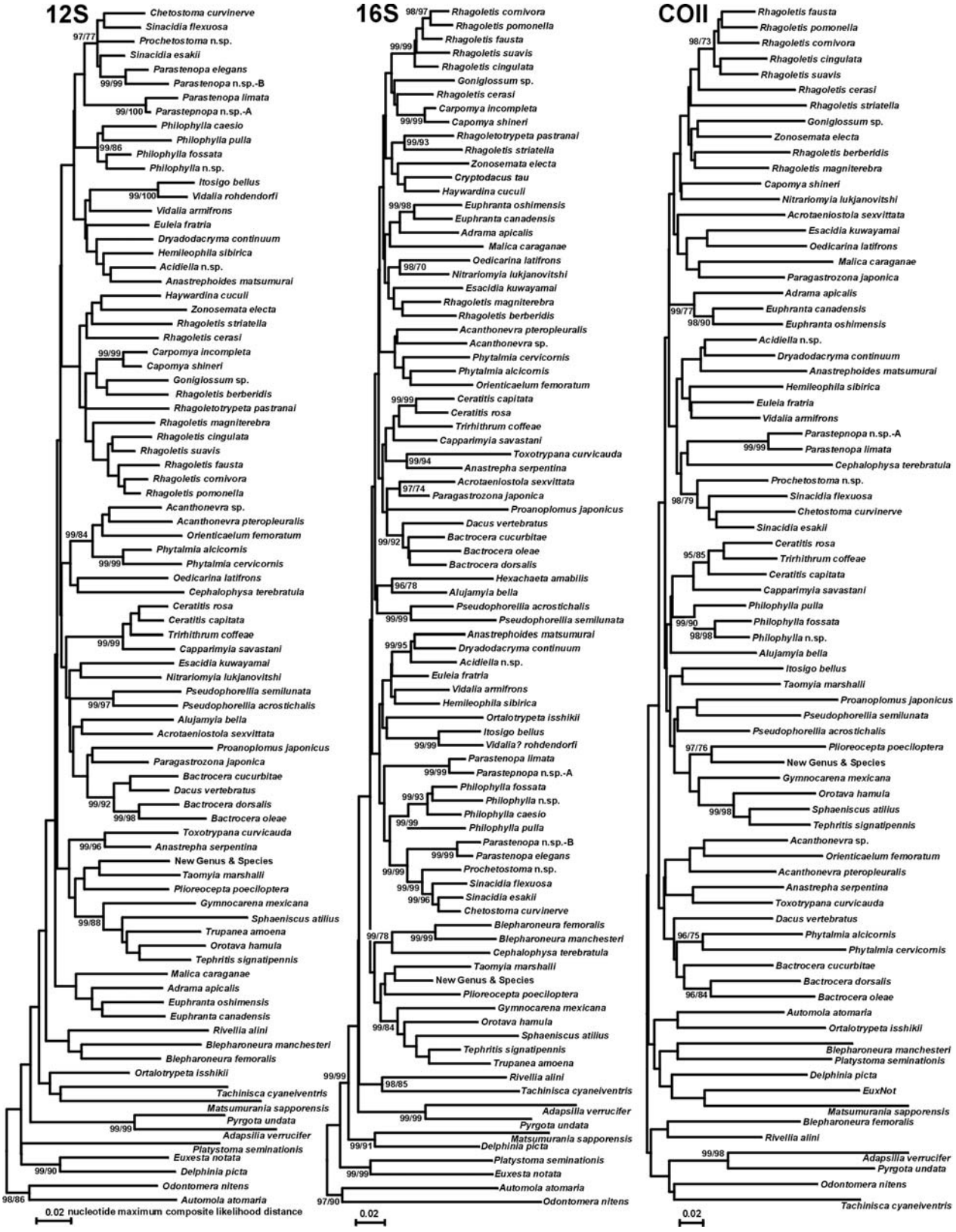

Fig. 1. Minimum evolution trees based on nucleotide maximum composite likelihood distances with pairwise deletion of gaps and missing data, using 12S rRNA (664 bp), 16S rRNA (1118 bp), and COII (669 bp) gene sequences. The first number on each branch is the Pc from the interior branch test, and the second number is the Pb from the bootstrap test (1000 replications). For the two rRNA datasets, ambiguously aligned sites were eliminated using Gblocks (parameter: default except allowed gap = with half).

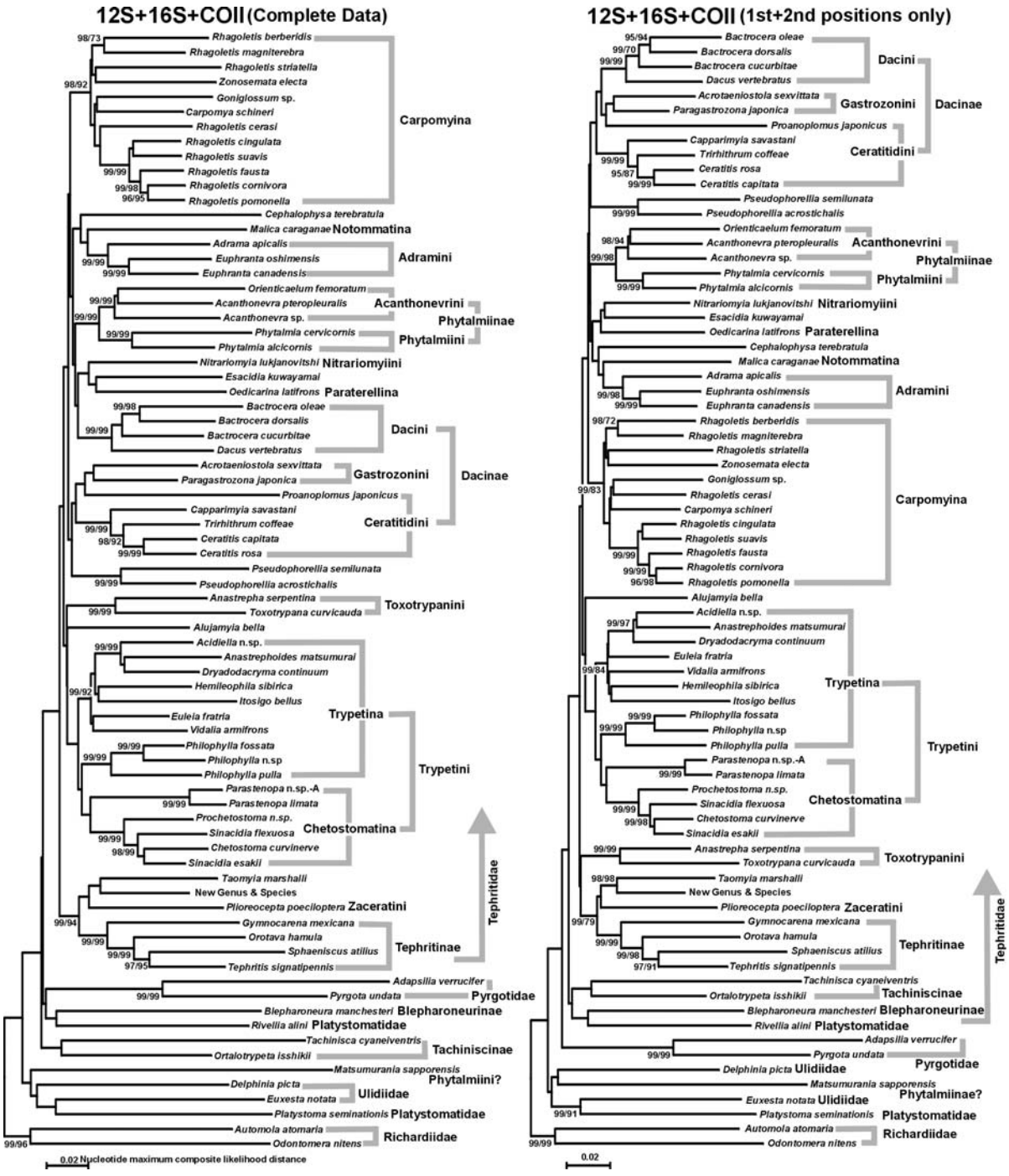

Fig. 2. Minimum evolution trees based on nucleotide maximum composite likelihood distances with pairwise deletion of gaps and missing data, using the combined sequences of 12S rRNA, 16S rRNA, and COII genes. We included either the complete COII gene (left-2451 bp) or the first and second codon positions from same gene (right-2228 bp). The first number on each branch is the Pc from the interior branch test, and the second number is the Pb from the bootstrap test (1000 replications). For the two rRNA datasets, ambiguously aligned sites were eliminated using Gblocks (parameter: default except allowed gap = with half).

additional species that diverged in the early evolutionary history of the family, or is simply an artifact.

Phylogenetic relationships within the family Tephritidae

A sister group relationship between *Ortalotrypeta* and *Tachinisca* is strongly supported in both ME and BI trees (Figs. 2 and 3). *Tachinisca* is a highly apomorphic taxon and a member of the group previously recognized as the separate family Tachiniscidae. Korneyev (1999a) indicated that the female terminalia of *Tachinisca* are extremely similar to the highly derived ovipositor

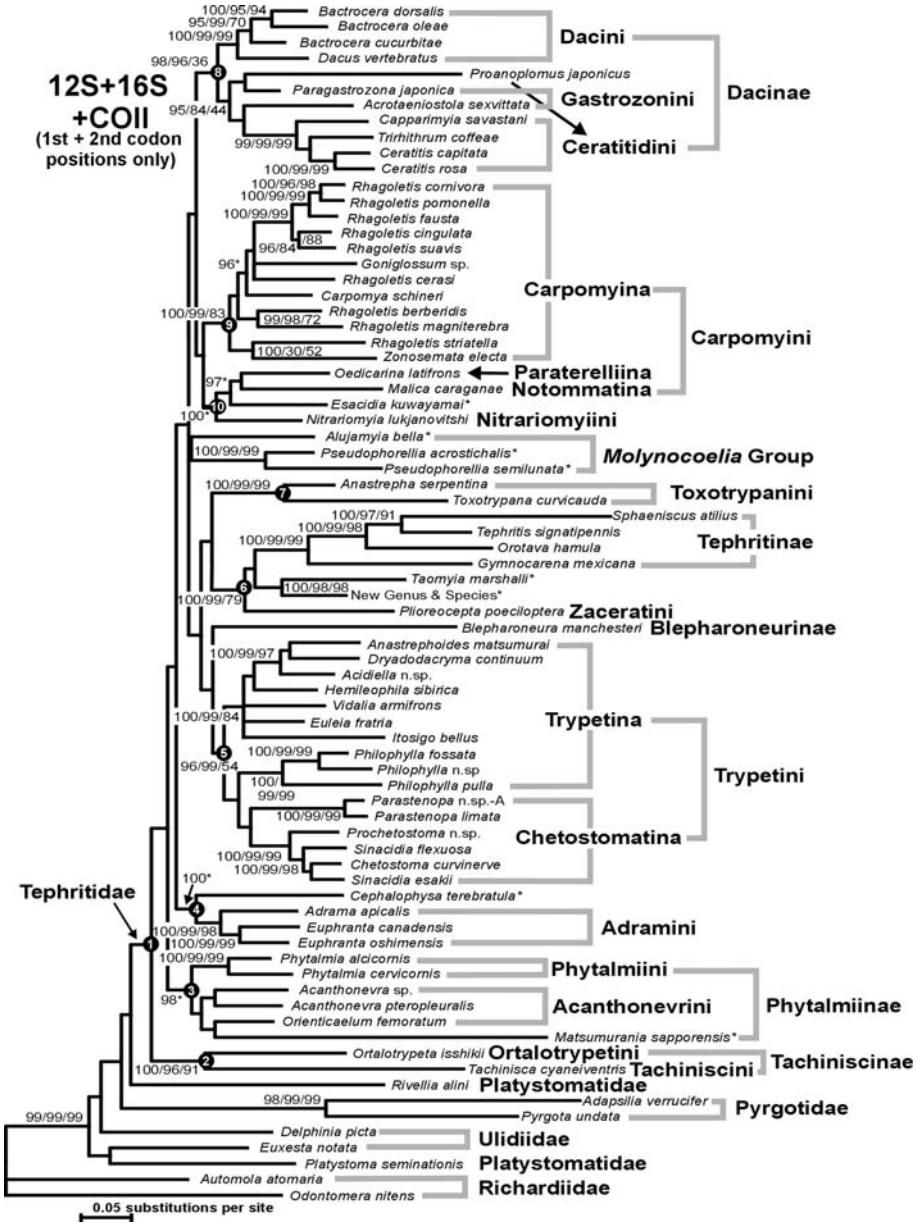

Fig. 3. The 50% majority rule consensus tree derived from Bayesian analysis of the combined sequences of 12S rRNA, 16S rRNA, and COII genes, using the GTR + I + G evolutionary model. The first number on each branch is the Pp from Bayesian analysis, the second number is the Pc from the interior branch test of the ME tree, and the third number is the Pb from the bootstrap test of the ME tree. Asterisks indicate the branches supported only by the Bayesian analysis.

structure of Ortalotrypetini, and therefore classified the tribes Tachiniscini and Ortalotrypetini under the tephritid subfamily Tachiniscinae. Korneyev (1999b) also listed six synapomorphies of the subfamily, but did not resolve the phylogenetic position of Tachiniscinae within the Tephritidae in the cladistic analysis. Korneyev suggested, however, that it may be the basal-most group within the Tephritidae, because of the rudimentary gonostyli observed in *Tachinisca* that is completely lost in other Tephritidae. More recently, Korneyev and Norrbom (2006) suggested that the absence of the greater ampulla indicates a sister group relationship between Tachiniscinae and the other tephritid subfamilies. Our data topologically, although not statistically, supports the basal phylogenetic position of the Tachiniscinae (Fig. 3-Node 2).

A sister group relationship between the Phytalmiini and Acanthonevrini is strongly supported in both ME and BI trees, but the inclusion of the enigmatic taxon *Matsumurania sapporensis* in the subfamily Phytalmiinae is supported only in the BI tree (Fig. 3-Node 3). A monotypic genus, *Matsumurania*, has often been placed in the Phytalmiinae because of its elongate appearance, which is superficially similar to some other subfamily members, but Korneyev (1999b) could not classify this genus based on the female characters alone.

Another monotypic genus, *Cephalophysa,* is currently also unplaced at the tribal level within the subfamily Trypetinae. Our BI analyses, however, strongly support its relationship to the tribe Adramini (Fig. 3-Node 4). The Adramini is currently defined as a monophyletic group based on a single synapomorphy: The presence of long, fine, erect setulae on the anatergite (Korneyev, 1999b). Because *Cephalophysa terebratula* might possibly be a sister group of the Adramini or a member of a tribe with that character reversed, we need more sampling of taxa and genetic markers to clarify its phylogenetic position.

The monophyly of Trypetini is strongly supported (Fig. 3-

Node 5), as suggested by our previous morphological and molecular studies (Han, 1992; 1999; 2000; Han and McPheron, 1997; 1999; Han and Norrbom, 2008). In the present study, however, the monophyly of the subtribe Trypetina is not supported, and it is not clear whether the Trypetina is a paraphyletic group or a monophyletic group not resolved by our analysis. In addition, we found that the previously unclassified genus *Sinacidia* of the subfamily Trypetinae actually belongs to the subtribe Chetosomatina and is likely to be congeneric with *Chetostoma*.

Our earlier studies (Han et al., 2006; Han and McPheron, 1997) suggested the monophyly of the subfamily Tephritinae and its sister group relationship with the genus *Plioreocepta*. The present study not only clearly supports the above relationships (Fig. 3-Node 6), but also unexpectedly places two taxa between these two lineages. The African genus *Taomyia* is currently unplaced at the tribal level within the heterogeneous subfamily Trypetinae (Norrbom et al., 1999). Interestingly, a putative new genus and species from Korea shows close affinity to *Taomyia* with exceptionally high statistical support values (Pp/Pc/Pb = 100/98/98). We examined their male genitalia and found that they also share the oval outline of epandrium and surstyli in posterior view that was hypothesized to be a synapomorphy of the Tephritinae and Zaceratini (including *Plioreocepta*) (Korneyev, 1999c). We believe that these newly discovered relationships are critical for phylogenetic analysis of the large subfamily Tephritinae (over 1800 known species), as they provide a more accurate root position of the tephritine lineage.

Han and McPheron (1997) suggested that the tribe Toxotrypanini and the genus *Hexachaeta* are closely related based on 16 rDNA sequences. Based on this result, Norrbom et al. (1999) included *Hexachaeta* as a member of the Toxotrypanini. Our present data based on an expanded sampling of taxa using the same genetic marker, however, do not support the same relationship (Fig. 1B). Instead, *Hexachaeta* is grouped with another New World genus, *Alujamyia*.

Norrbom (2006) grouped the Neotropical genera *Molynocoelia*, *Pseudophorellia*, and *Alujamyia* in the *Molynocoelia* group. He postulated that the *Molynocoelia* group and the Paleotropical genera *Callistomyia* and *Alincocallistomyia* might be closely related because they share a similar pattern of spicules on the eversible membrane and the usual presence of setulae on the katepimeron or dorsal margin of the meron. He further speculated, without certainty, that this clade might be related to the tribe Toxotrypanini based on the presence of similar wing patterns. Our molecular data lacked sequences for *Molynocoelia*, *Callistomyia*, and *Alincocallistomyia*, so we could demonstrate only that *Alujamyia* and *Pseudophorellia* are grouped together without significant statistical support (Figs. 1B and 3), and *Alujamyia* and *Hexachaeta* are grouped in the 16S tree (Fig. 1B; see also the preceding paragraph). An alternative hypothesis of Norrbom's (2006) was that the *Molynocoelia* group, *Callistomyia*, and *Alincocallistomyia* are more closely related to the Adramini based on their unusual katepimeral setulae. Our data, however, do not support these relationships, which will require additional sampling of taxa and genetic markers to be resolved.

Our data clearly suggest that the subfamily Dacinae is monophyletic (Fig. 3-Node 8) but the subtribes Gastrozonini and Ceratitidini may not be. Korneyev (1999b) also mentioned that the Gastrozonini may not be monophyletic, and further suggested that the Ceratitidini should be included within the Gastrozonini. Our results appear to support this suggestion, but, unfortunately, our sampling of this large subfamily was limited. We need more extensive sampling of taxa to resolve their intertribal relationships.

Korneyev (1996; 1999b) tentatively placed the subtribes Carpomyina, Notommatina, and Paraterelliina in the tribe Carpomyini. Without any significant statistical support, the BI tree (Fig. 3) topologically recognizes a group including the Carpomyini, the Nitrariomyini, and the unclassified genus *Esacidia*. Within this group, two major monophyletic clades, the subtribe Carpomyina (Fig. 3-Node 9) and the rest of the taxa (Fig. 3-Node 10), are recognized based on strong statistical support. The latter group has never been recognized as a monophyletic clade, so this classification needs to be tested by additional sampling of taxa. In contrast, the monophyly of the subtribe Carpomyina has been repeatedly suggested based on previous morphological and molecular studies (Jenkins, 1996; Han and McPheron, 1997, 1999; Korneyev, 1999b; Smith and Bush, 1999; Smith et al., 2006). Within this subtribe, the nonmonophyletic nature of the widely distributed genus *Rhagoletis* is also recognized. As more data accumulate, the status of this genus should be extensively revised.

Taxonomic implications

Our results are highly compatible with the relatively well-established phylogenetic hypotheses involving tephritid subfamilies and tribes. We therefore believe that combined analysis of the 12S, 16S, and COII gene sequences are useful for delimiting higher relationships and classifying many taxa with uncertain relationships. For example, we were able to place the genus *Sinacidia* in the subtribe Chetostomatina, and found that the genus *Matsumurania* possibly belongs in the tribe Acanthonevrini and *Cephalophysa* in the Adramini. We also generated new data that might improve higher tephritid classifications. For example, our results show that the genera *Plioreocepta*, *Taomyia*, and an unnamed new genus are closely related to the subfamily Tephritinae. This is critical for the phylogenetic analysis and classification of this large but relatively well-defined subfamily.

Because there are many unclassified tephritid genera in the current accepted classification of Norrbom et al. (1999), it is possible that some major phylogenetic lineages of the Tephritidae were not even represented in our dataset. We need, in the future, to sample as many unclassified tephritid taxa as possible in addition to the tribes not represented in our analysis (i.e., Epacrocerini, Phascini, Rivelliomimini, and Xarnutini). Our previous study indicated that adding gene regions to the dataset provided better phylogenetic resolution among tephritoid taxa (Han and Ro, 2005). Inclusion of gene regions with similar levels of variability (e.g., COI and COIII genes) will be needed for finer resolution of the deep phylogenetic branches within the family Tephritidae.

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