



Phylogenetic relationships of the tribe Toxotrypanini (Diptera: Tephritidae) based on molecular characters



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ABSTRACT

Current hypotheses of relationship among the species of the fruit fly genera *Anastrepha* and *Toxotrypana* are tested using sequence data from six DNA regions: the mitochondrial regions 16S, CAD, and COI, and the nuclear regions EF1a, PER, and PGD. DNA sequences were obtained from 146 species of *Anastrepha*, representing 19 of the 21 species groups as well as five of the six clades of the *robusta* group, and four species of *Toxotrypana* in addition to species of *Hexachaeta*, *Pseudophorellia*, *Alujamyia*, and 13 other tephritid genera used as outgroups. The results indicate that *Hexachaeta* is more closely related to the *Molynocoelia* group than to *Toxotrypana* and *Anastrepha*, and it is removed from the tribe Toxotrypanini. The group *Anastrepha* + *Toxotrypana* and the genus *Toxotrypana* are strongly supported as monophyletic, consistent with previous studies, but *Toxotrypana* arises within *Anastrepha*, confirming that *Anastrepha* as currently defined is paraphyletic. The placement of *Toxotrypana* within *Anastrepha* is clearly defined for the first time with high support, as the sister group to the *cryptostrepha* clade of the *robusta* group of *Anastrepha*. Within *Anastrepha*, the *daciformis*, *dentata*, *leptozona*, *raveni*, and *striata* species groups are highly supported clades. The *serpentina* group is recognized with lower support, and the *fraterculus* and *pseudoparallela* groups are supported with minor alterations. The *robusta* group is resolved as polyphyletic, but four of the six species clades within it are recovered monophyletic (one clade is not represented and another is represented by one species). The *punctata* and *panamensis* groups are resolved together in a clade. At least some species of the *mucronota* group are related, however this group requires further study. The *benjamini*, *grandis*, and *spatulata* groups appear to be polyphyletic. Relationships among the species groups are generally poorly resolved, with the following exceptions: (1) the lineage including *Toxotrypana*, the *cryptostrepha* clade, and the *tripunctata* group; (2) the sister group relationship of the *daciformis* + *dentata* groups; (3) a clade comprising the *punctata* and *panamensis* groups; and (4) the large clade comprising the *pseudoparallela* + *spatulata* + *ramosa* + *grandis* + *serpentina* + *striata* + *fraterculus* groups.

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1. Introduction

The true fruit flies (Tephritidae) comprise one of the most diverse families of the Order Diptera, with more than 4900 described species (Norrbom, unpubl. data). This family includes

major pests of many fruit and vegetable crops and is the most agriculturally important family of flies (White and Elson-Harris, 1992; Norrbom, 2010).

With more than 300 species, *Anastrepha* Schiner and *Toxotrypana* Gerstaecker form the largest clade of fruit flies in the New World (Norrbom et al., 1999a, 1999b, 2015; Norrbom, 2004a; Norrbom and Korytkowski, 2009, 2011, 2012). *Anastrepha* includes nearly 300 described species and *Toxotrypana* includes seven, but new species are currently being described (Norrbom et al., 2012, 2014, 2015) and numerous additional undescribed species of both genera are known (Norrbom, unpublished data). These genera also include the most important pest fruit fly species in the Neotropics, such as the Mexican fruit fly (*Anastrepha ludens* (Loew)), the West Indian fruit fly (*Anastrepha obliqua* (Macquart)), the South American fruit fly (*Anastrepha fraterculus* complex), and the papaya fruit fly (*Toxotrypana curvicauda* Gerstaecker), impacting major commercial and subsistence crops such as mango, citrus, guava, papaya and many others (Norrbom, 2004b).

Despite their importance to agriculture and the great diversity of these flies, the phylogenetic relationships within the *Anastrepha*/*Toxotrypana* clade are poorly understood. Together these two genera form a well-defined monophyletic group supported by both morphological (Norrbom et al., 1999b) and molecular studies (Han and McPheron, 1997; McPheron et al., 1999; Segura et al., 2006; Han and Ro, 2009). Based on previous studies, *Toxotrypana* also is clearly monophyletic, but *Anastrepha* may be paraphyletic; the exact relationships among *Toxotrypana* and the various species groups of *Anastrepha* are uncertain (McPheron et al., 1999; Norrbom et al., 1999b; Barr et al., 2005). Both genera are classified in the tribe Toxotrypanini (subfamily Trypetinae), which also includes *Hexachaeta* Loew (Norrbom et al., 1999b).

Anastrepha has been divided into various species groups based on morphological characters (Norrbom and Kim, 1988; Norrbom et al., 1999b, 2012; Norrbom and Korytkowski, 2009). Norrbom et al. (2012) recognized 21 species groups, one of which (the *robusta* group) included six clades (Norrbom and Korytkowski 2009). Norrbom (1997) also recognized the *pallidipennis* complex within the *pseudoparallela* group, and Norrbom and Korytkowski (2012) and Norrbom et al. (2015) recognized the *megacantha* and *lanceola* clades within the *mucronota* group. Except within a few species groups (Norrbom, 1998, 2002; Norrbom and Korytkowski 2009), there have been no rigorous phylogenetic analyses based on morphological characters, although Norrbom et al. (1999b) listed putative synapomorphies for some species groups and indicated the basis for others. Many of the morphological characters useful for species diagnosis, such as terminalia length and the shape of the aculeus tip, intergrade and are thus difficult to use in phylogenetic analysis.

Molecular studies to test the monophyly of and relationships among the species groups of *Anastrepha* have been very limited (Silva and Barr, 2008). McPheron et al. (1999) analyzed the relationships among 43 species of *Anastrepha* and *Toxotrypana* based on mitochondrial 16S rRNA, and Barr et al. (2005) reanalyzed most of these species using part of the nuclear protein-coding gene *period*. Smith-Caldas et al. (2001) analyzed the relationships among 15 *Anastrepha* species, mainly within the *fraterculus* group, based on the mitochondrial protein-coding gene cytochrome *c* oxidase subunit I (COI), and Segura et al. (2006), using mitochondrial DNA encompassing the *cytb*, *tRNA^{Ser}* and *ND1* genes, studied six species of *Anastrepha* and *Toxotrypana*. Ruiz et al. (2007a, 2007b) surveyed the relationships among 12 species based on sequences of the sex-determination nuclear genes *doublesex* (*dsx*) and *transformer* (*tra*). And in a study focused on *A. obliqua* (Macquart), Scally et al. (2016) analyzed relationships among ten species, mostly in the *fraterculus* group, based on seven nuclear and two mitochondrial loci. Although these preliminary investigations are

important contributions, their limited dimensions in terms of taxa and characters and low support values for most clades provide few competing hypotheses of relationship.

The relationships of *Hexachaeta*, the third genus in the Toxotrypanini, and those of the Toxotrypanini with other tribes of Trypetinae are poorly understood. *Hexachaeta* includes 28 described and at least 8 undescribed species ranging from southern Texas (USA) to northern Argentina and the Greater Antilles (Jamaica) (Hernández-Ortiz, 2006). Hernández-Ortiz (2006) analyzed the phylogenetic relationships within *Hexachaeta* based on morphological characters. He divided it into two subgenera, each with two species groups: *Hexachaeta s. str.*, including the *colombiana* and *eximia* species groups; and *Costamyia* Hernández-Ortiz, including the *amabilis* and *socialis* species groups. Hancock (1986) followed Foote (1980) and suggested to keep this genus in the tribe Trypetini. Korneyev (1994) placed *Hexachaeta* in the monotypic tribe Hexachaetini of the subfamily Xarnutinae, which is not currently recognized. Korneyev (1999) later included the Australasian genera *Alincocalistomyia* Hardy and *Callistomyia* Bezzi in the Hexachaetini within the subfamily Trypetinae. Based on analysis of 16S sequences, Han and McPheron (1997) hypothesized *Hexachaeta* as the possible sister group of *Anastrepha* + *Toxotrypana*, and on this basis Norrbom et al. (1999a, 1999b) included *Hexachaeta* in the Toxotrypanini. Norrbom (2006) performed a morphological phylogenetic analysis of the *Molynocoelia* group, which includes the genera *Alujamyia* Norrbom, *Molynocoelia* Giglio-Tos, and *Pseudophorellia* Lima. In his work, Norrbom (2006) suggested that those genera may be closely related to those previously included in the Hexachaetini and Toxotrypanini and/or the Adramini.

The aim of the present work is threefold: first, to infer the phylogenetic relationships of the Toxotrypanini, including *Anastrepha*, *Toxotrypana* and *Hexachaeta*, using molecular characters; second, to test the monophyly of *Anastrepha* or its paraphyly with respect to *Toxotrypana*; and last, to test the monophyly of and to infer the relationships among the species groups and subclades of *Anastrepha*. To accomplish these objectives, six DNA regions were sequenced and analyzed: part of the mitochondrial 16S rRNA gene; the 5'-region of the carbomoylphosphate synthase (CPS) domain of the nuclear *rudimentary* gene (CAD) (aminoacids 54–405); the entire mitochondrial protein-coding gene cytochrome *c* oxidase subunit I (COI); part of the nuclear protein-coding gene elongation factor 1- α (EF1 α); part of the nuclear protein-coding gene *period* (PER); and part of the nuclear protein-coding gene 6-phosphogluconate dehydrogenase or *pgd* (PGD).

2. Material and methods

2.1. Taxonomic sampling

A full list of included taxa is provided in Table 1. Vouchered specimens are identified with unique specimen identifier numbers following lab and/or institutional convention. For instance, USNM specimens have USNM codes. Some specimens have multiple identifiers or laboratory codes because the voucher specimen is deposited in one institution, but DNA was extracted at another laboratory. Codes starting with a year + PHK or with 6 digit numbers starting with 0 and a letter in third position pertain to specimens whose DNA extractions were done at the California Department of Food and Agriculture (CDFA). Codes starting with PSU originated in the McPheron laboratory at Pennsylvania State University; additional DNA regions were sequenced in the APHIS laboratory (Edinburg) from extractions of these specimens. Codes starting with a V or TBI pertain to the APHIS lab. To avoid confusion but to maintain traceability of the specimens, unique identifier codes for individual

Table 1

Taxon sampling used in the molecular analysis listed in alphabetic order, including GenBank accession numbers. All GenBank accession numbers starting with KY42 denote new sequences used for the first time in the present study. Composite taxa are indicated in bold.

Taxon	Species group	Identifier/Depository collection	COI 5' (1490–2198)	COI 3' (2195–3014)	16 S	PGD	EF-1a	PERIOD	CAD (54–410)	Specimen data
<i>Acinia picturata</i> (Snow)		USNMENT00875647 (07Y648)/USNM	KY428226	KY428226	KY427949				KY428106	USA: Florida, Miami-Dade Co., Kendall, USDA Subtropical Agricultural Research Center, 25°38'35.9"N 80°17'38.9"W, 19. May.2004, P. H. Kerr; male; det. A.L. Norrbom
<i>Alujamyia bella</i> Norrbom		USNMENT00215041/USNM	KY428229	KY428229	KY427952				KY428109	MEXICO: Veracruz, Apazapan, 19°19'N 96°43'W, McPhail trap 2 in chico zapote orchard, 28.Feb.2002, M. Aluja; ALN-2002-85; paratype male; det. A. L. Norrbom
<i>Alujamyia isolata</i> Norrbom		USNMENT00671883/USNM	KY428237	KY428237	KY427960			KY428510		PUERTO RICO: Loiza, Rt. 187, Municipal cemetery, fruit fly trap in <i>Terminalia catappa</i> , 9.Jul.2009, R. Hernandez; male; det. A. L. Norrbom
<i>Anastrepha aberrans</i> Norrbom	<i>grandis</i>	USNMENT00215483 (2004PHK013)/USNM	KY428323	KY428323	KY428037				KY428166	PANAMA: Altos de Pacora, 14.Aug.1997, C. A. Korytkowski, McPhail trap 547; male; det. C. A. Korytkowski
<i>Anastrepha acris</i> Stone	<i>fraterculus</i>	V080807-1 (PSU-000427-9)/-		AF420625	AF152049				AY532486	VENEZUELA: Falcón, Boca del Tocuyo, reared from <i>Hippomane mancinella</i> , 7. May.1993, K. Katiyar & R. Matheus. (McPheron et al., 1999; Smith-Caldas et al., 2001; Barr et al., 2005)
<i>Anastrepha adami</i> Norrbom	unknown	USNMENT00744867/USNM	KY428386	KY428386	KY428084		KY428493		KY428198	PERU: Cusco, Estación Biológica Villa Carmen, trail 8, mark 8-2024, 12.90203°S 71.41135°W, 760 m, trap VC-ML-17A, 24–30.Apr.2013, M. Choque; paratype female; det. A. L. Norrbom
<i>Anastrepha alveata</i> Stone	<i>spatulata</i>	V080807-2/-			AF152050					MEXICO: Veracruz, Llano Grande Ravine, ex. <i>Ximania americana</i> , 1994, M. Aluja. (McPheron et al., 1999)
		USNMENT00745035/USNM	KY428248	KY428248					KY428516	PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°12'38.8"N 79°22'37.3"W, 814 m., trap II UA2, 30. Jun.2009, E. J. Rodriguez; male; det. C. A. Korytkowski
		USNMENT00213712 (05V857)/USNM							KY428121	COSTA RICA: Guanacaste, near Liberia, 12. Mar.2003, Sanidad Vegetal, McPhail trap; female; det. A. L. Norrbom
<i>Anastrepha amita</i> Zucchi	<i>fraterculus</i>	06X646 (V080807-3)/-	KY428324	AF420626	AF152051					TRINIDAD: Victoria Parish, Oct–Nov.1991, K-80 and K-88; det. A. L. Norrbom. (McPheron et al., 1999; Smith-Caldas et al., 2001)
<i>Anastrepha ampliata</i> Hernández-Ortiz	<i>fraterculus</i>	USNMENT00875785 (2004PHK015)/USNM	KY428325	KY428325	KY428038					GUATEMALA: Ecuintla, Palín, Finca Silmar, 1.Oct.2001, #4; ALN-2002-71; female; det. A. L. Norrbom
<i>Anastrepha amplidentata</i> Norrbom	<i>fraterculus</i>	USNMENT00671842/USNM	KY428264	KY428264	KY427984		KY428435		KY428132	PERU: Madre de Dios, Tambopata, Las Piedras, Planchón, trampa 1.2.1.1, 8. Dec.2006, B. Sacachipana 0250-07; paratype female; det. A. L. Norrbom
<i>Anastrepha annonae</i> Norrbom	previously <i>fraterculus</i> , here as unknown	USNMENT00744776/USNM	KY428390	KY428390	KY428088	KY428642	KY428497		KY428202	PERU: Cusco, Estación Biológica Villa Carmen, trail 4, next to airplane, 12.89341°S 71.40557°W, 543 m, emerged 23–25.Feb.2013 reared from fruit of

<i>Anastrepha anomala</i> Stone	<i>serpentina</i>	USNMENT00212784 (06X521)/ USNM	KY428326	KY428326	KY428039					<i>Annona mucosa</i> Jacq. (VC-10) collected Jan.2013, E. J. Rodriguez; paratype female; det. A. L. Norrbom PANAMA: Altos de Pacora, Desvio, McPhail Trap 520, 9Jul.2004, C. A. Korytkowski; ALN-2006-28B; male; det. A. L. Norrbom BOLIVIA: Santa Cruz, El Torno, 09Jul.2001, McPhail trap; female; det. A. L. Norrbom PERU: Tingo Maria area, [no date], G. Egoavil, M003; female; det. A. L. Norrbom PUERTO RICO: Ponce, Adjuntas, Rd. 135, km. 82.0 interior, Jul.2002, L. Sotomayor & W. Feliciano, trap 135-04; ALN-2003-33; male; det. A. L. Norrbom PUERTO RICO: Mayagüez: S of Maricao, Road 120, km. 14.8, 18.17°N 66.975°W, McPhail trap 120-09 in mango, 29 Mar 2007, I. M. Perez & E. O. Rivera; male; det. A. L. Norrbom PUERTO RICO: Rd. 120, km. 18, trap 120- 10, 6-20.Mar.2006, Ramirez & Perez; ALN- 2006-58; male; det. A. L. Norrbom BRAZIL: Espírito Santo, Linhares, Atlantic rain forest, 24-30.Apr.2003, K. Uramoto; female; det. K. Uramoto MEXICO: Veracruz: Pozarica, em. 18. Oct.1997, M. Aluja et al. (McPheron et al., 1999; Barr et al., 2005) PERU: Cajamarca, Jesús, Chuco, 8. Feb.2010, Trampa McPhail, E. Gaitán; female; det. A. L. Norrbom PANAMA: Altos de Pacora, McPhail Trap, 2006, C. A. Korytkowski; ALN-2006-22; female; det. A. L. Norrbom COSTA RICA: San José, San Isidro del General, vicinity of, 9°25.72'N 83°42.61'W, 1189 m, 17.Apr.2007, D. Thomas & D. Robacker; female; det. A. L. Norrbom GUATEMALA: Taxisco, 1994. (Barr et al., 2005) PERU: Madre de Dios, Estación Biológica Villa Carmen, trail 13, near high point, 12.86781°S 71.42547°W, 713 m, trap VC- ML-36, 3-9.Feb.2013, E. J. Rodriguez; female; det. A. L. Norrbom BOLIVIA: Santa Cruz: Florida, 4 km N of Bermejo, Refugio Los Volcanes, 18°6'S 63°36'W, 1037-1280 m, 16-20 Sep 2012, P. Skelley, J. Wappes, T. Bonaso & C. Hammel; female; det. A. L. Norrbom BRAZIL: Minas Gerais, Arceburgo, 1991. (Barr et al., 2005) PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°12'38.8"N 79°22'37.3"W, 814 m., trap II 9UA2, 13. Nov.2009, E. J. Rodriguez; male; det. C. A. Korytkowski
Anastrepha anomoiiae Norrbom	<i>serpentina</i>	USNMENT00671870/USNM	KY428249	KY428249		KY428597		KY428517		
Anastrepha antilliensis Norrbom	<i>daciformis</i>	USNMENT00214418 (2004PHK016)/USNM USNMENT00213996 (05V841)/ USNM			KY427970			KY428040		
		USNMENT00212132 (TBI- V080416-19)/USNM							KY428565	
		USNMENT00875650 (06X797)/ USNM	KY428327	KY428327						
<i>Anastrepha antunesi</i> Lima	<i>fraterculus</i>	USNMENT00875725 (05V050)/ USNM	KY428250	KY428250	KY427971				KY428122	
<i>Anastrepha</i> <i>aphelocentema</i> Stone	<i>mucronota</i>	V080807-4/-	KY428328		AF152053				AY532488	
<i>Anastrepha atrox</i> (Aldrich)	<i>mucronota</i>	USNMENT00671955/USNM	KY428251	KY428251	KY427972	KY428598	KY428424		KY428123	
<i>Anastrepha avispa</i> Norrbom	<i>daciformis</i>	USNMENT00875744 (07y640)/ USNM	KY428329	KY428329	KY428041					
Anastrepha bahiensis Lima	<i>fraterculus</i>	USNMENT00671597/USNM	KY428252	KY428252	KY427973	KY428599	KY428425		KY428124	
		V080807-5/-							AY532488	
<i>Anastrepha bahiensis</i> Lima	<i>fraterculus</i>	USNMENT00744880/USNM	KY428372	KY428372	KY428072	KY428631	KY428479		KY428184	
Anastrepha barbiellinii Lima	unknown	USNMENT00744924/USNM	KY428373	KY428373	KY428073	KY428632	KY428480		KY428185	
									AY532490	
<i>Anastrepha barnesi</i> Aldrich	<i>leptozonea</i>	USNMENT00745025/USNM	KY428253	KY428253	KY427974				KY428518	

(continued on next page)

Table 1 (continued)

Taxon	Species group	Identifier/Depository collection	COI 5' (1490–2198)	COI 3' (2195–3014)	16 S	PGD	EF-1a	PERIOD	CAD (54–410)	Specimen data
<i>Anastrepha bella</i> Norrbom & Korytkowski	previously <i>robusta</i> , here as unknown	USNMENT00875783 (2004PHK183)/USNM	KY428330	KY428330	KY428042					PANAMA: Cerro Jefe, 5.Sep.1997, C. A. Korytkowski; ALN-2002-45; male; det. C. A. Korytkowski
<i>Anastrepha bezzii</i> Lima	<i>mucronota</i>	USNMENT00745006/USNM	KY428254	KY428254	KY427975	KY428600	KY428426	KY428519	KY428125	PANAMA: Panamá, Parque Nacional Chagres, Cerro Azul, Escuela, 9°8'9.3"N 79°23'29.8"W, Escuela, 323 m, trap III 3UM 1, 8.Apr.2010, E. J. Rodriguez; female; det. E. J. Rodriguez
<i>Anastrepha bicolor</i> (Stone)	<i>daciformis</i>	PSU-971210-02/-	KY428331	KY428331	AF152058			AY532493		MEXICO: Veracruz, Emiliano Zapata, La Jicayana, Feb.1996, P. Juárez. (McPheron et al., 1999; Barr et al., 2005)
<i>Anastrepha bistrigata</i> Bezzi	<i>striata</i>	PSU-6-20-94-3/-	KY428332		AF152059			AY532494		BRAZIL: São Paulo, Louveira, from lab colony of J. S. Morgante and A. Malavasi, Dept. de Biol., USP, São Paulo, 1990, G. J. Steck. (McPheron et al., 1999; Barr et al., 2005)
<i>Anastrepha bivittata</i> (Macquart)	<i>grandis</i>	USNMENT00875732 (05V054)/USNM	KY428340	KY428340	KY428047				KY428171	BRAZIL: Espírito Santo, Atlantic rain forest, Linhares, 12–18.Mar.2004, K. Uramoto; female; det. A. L. Norrbom
<i>Anastrepha breviapex</i> Norrbom	previously <i>mucronota</i> , here as unknown	USNMENT00744592/MHNJP	KY428362	KY428362			KY428478	KY428571	KY428180	PERU: Cusco, Echarate, Urusayhua, May.2012, T. Guevara; holotype female; det. A. L. Norrbom
<i>Anastrepha buscki</i> Stone	previously unknown, here placed in <i>pseudoparallela</i>	USNMENT00744881/USNM	KY428375	KY428375	KY428075		KY428482		KY428187	PERU: Cusco: Estación Biológica Villa Carmen, trail 8, mark 8-1952, 12°54'8"S 71°24'38"W, 718 m, trap VC-ML-17, 13–23.Apr.2013, M. Choque; female; det. A. L. Norrbom
<i>Anastrepha caballeroi</i> Norrbom	<i>mucronota</i>	USNMENT00104984/USNM	KY428405	KY428405	KY428100				KY428215	PERU: Madre de Dios, Estación Biológica Villa Carmen, trail 13, near high point, 12.86781°S 71.42547°W, 713 m, trap VC-ML-36, 24–30.Jan.2013, E. J. Rodriguez; paratype female; det. A. L. Norrbom
<i>Anastrepha camba</i> Norrbom	unknown	USNMENT00671844/SENASA	KY428403	KY428403	KY428099					PERU: Junín, Chanchamayo, Pichanaki, Villa Santa María, trampa 1.6.7.1, 21. Oct.2008, G. González I. 0100-08; paratype female; det. A. L. Norrbom
<i>Anastrepha canalis</i> Stone	<i>fraterculus</i>	USNMENT00671618/USNM	KY428256	KY428256	KY427977	KY428601	KY428428	KY428520		COSTA RICA: San José, San Isidro del General, vicinity of, 9°25.72'N 83°42.61'W, 1189 m, 17.Apr.2007, D. Thomas & D. Robacker; female; det. A. L. Norrbom
<i>Anastrepha canalis</i> Stone	<i>fraterculus</i>	USNMENT00671619/USNM	KY428257	KY428257	KY427978	KY428602	KY428429	KY428521	KY428126	COSTA RICA: San José, San Isidro del General, vicinity of, 9°25.72'N 83°42.61'W, 1189 m, 17.Apr.2007, D. Thomas & D. Robacker; male; det. A. L. Norrbom
<i>Anastrepha chichlayae</i> Greene	<i>pseudoparallela</i>	USNMENT00875741/USNM	KY428263	KY428263	KY427983		KY428434	KY428526	KY428131	PERU: Piura, Alto Piura, La Matanza, Carrasquillo, trap 02,03,04,0027P, 25. Feb.2008; det. N. Nolzco
<i>Anastrepha cicra</i> Norrbom	<i>fraterculus</i>	USNMENT00744875/MHNJP	KY428389	KY428389	KY428087	KY428641	KY428496		KY428201	PERU: Cusco, Estación Biológica Villa Carmen, trail 8, mark 8-1747, 12.90241°S 71.40897°W, 723 m, trap VC-ML-15A, 3–9.Aug.2013, M. Choque; holotype female; det. A. L. Norrbom

<i>Anastrepha cocorae</i> Norrbon & Korytkowski	<i>hastata</i>	USNMENT00671689/USNM	KY428258	KY428258	KY427979	KY428603	KY428430	KY428522		PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°13'36"N 79°21'54"W, trap I UF 1, Jun–Aug.2009, E. J. Rodriguez & C. A. Korytkowski; female; det. C. A. Korytkowski
<i>Anastrepha cocorae</i> Norrbon & Korytkowski	<i>hastata</i>	USNMENT00745012/USNM	KY428259	KY428259	KY427980	KY428604	KY428431	KY428523	KY428127	PANAMA: Panamá: Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap I 24 UP2, 30. Jun.2009, E. J. Rodriguez; male; det. C. A. Korytkowski
<i>Anastrepha compressa</i> Stone	<i>fraterculus</i>	USNMENT00745041/USNM	KY428260	KY428260	KY427981	KY428605	KY428432	KY428524	KY428128	PANAMA: Panamá, Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap I 9 BM1, 22. Oct.2009, E. J. Rodriguez; female; det. C. A. Korytkowski
<i>Anastrepha concava</i> Greene	previously <i>robusta</i> , here as unknown	USNMENT00212059 (08A300)/ USNM								BOLIVIA: Cochabamba, Chapare, Agrigento A, trampa McPhail R3-3, 17.Jun.2005, E. Quisberth; female; det. A. L. Norrbom
		USNMENT00744884/USNM	KY428376	KY428376			KY428483	KY428574	KY428188	PERU: Cusco, Estación Biológica Villa Carmen, trail 8 mark 8-1799, 12°54'9"S 71°24'34"W, 700 m, trap VC-ML-18, 27. Jul–2.Aug.2013; male; det. A. L. Norrbom
<i>Anastrepha consobrina</i> (Loew)	<i>pseudoparallela</i>	USNMENT00875731 (05V053)/ USNM	KY428333	KY428333	KY428043				KY428167	BRAZIL: Espírito Santo, Alfredo Chaves, Fecafundó farm, 15–21.Mar.2002, K. Uramoto; female; det. K. Uramoto
<i>Anastrepha cordata</i> Aldrich	<i>robusta, cryptostrepha</i> clade	V080807-9 (06X653)/–	KY428334	KY428334	AF152060			AY532495		MEXICO: Veracruz, Los Tuxtlas, Coyame-Nancyaga, ex <i>Tabernaemontana alba</i> , 1994, M. Aluja et al. (McPheron et al., 1999; Barr et al., 2005)
<i>Anastrepha coronilli</i> Carrejo & González	<i>fraterculus</i>	USNMENT00875648 (07Y424)/ USNM	KY428261	KY428261					KY428129	COLOMBIA: Santander, Bucaramanga, McPhail trap in <i>Mangifera indica</i> , 24. April.2006, F. Arias; ALN-2006-93; female; det. A. L. Norrbom
		V080807-10/–			AF152061			AY532496		VENEZUELA: Palmichal, 1.May.1993, K. Katiyar (McPheron et al., 1999; Barr et al., 2005)
<i>Anastrepha crebra</i> Stone	<i>mucronota</i>	05CA008/–	KY428335	KY428335					KY428168	GUATEMALA: Peña Plata, 5.Dec.2001, Lopez #23; ALN-2002-62; female; det. A. L. Norrbom,
		891722 043 (V080807-11)/–			AF152062			AY532497		MEXICO: Veracruz, Estación Biología Los Tuxtlas, ex. fruits of <i>Quararibea funebris</i> , 22.Aug.1989, V. Hernández & A. L. Norrbom. (McPheron et al., 1999; Barr et al., 2005)
<i>Anastrepha cryptostrepha</i> Hendel	<i>robusta, cryptostrepha</i> clade	USNMENT00744886/USNM				KY428484	KY428634			PERU: Madre de Dios, Estación Biológica Villa Carmen, trail 1 to waterfall, 12.87172°S 71.42737°W, 570 m, trap VC-ML-31, 23–30.Jan.2013, E. J. Rodriguez; female; det. A. L. Norrbom
		USNMENT00744887/USNM	KY428377	KY428377	KY428077				KY428189	PERU: Cusco, Estación Biológica Villa Carmen, trail 8, 12.90219°S 71.41039°W, 724 m, trap VC-ML-16A, 24–30.Apr.2013, M. Choque; female; det. A. L. Norrbom
<i>Anastrepha cryptostrephoides</i> Norrbon & Korytkowski	<i>robusta, cryptostrepha</i> clade	USNMENT00744114/USNM	KY428262	KY428262	KY427982		KY428433	KY428525	KY428130	PERU: Cusco, Echarate region, Mar–Dec.2011, T. Guevara; female; det. A. L. Norrbom
<i>Anastrepha curitis</i>	<i>pseudoparallela</i> ,	USNMENT00744888/USNM	KY428378	KY428378	KY428078	KY428635	KY428485	KY428575	KY428190	PERU: Madre de Dios, Centro de

(continued on next page)

Table 1 (continued)

Taxon	Species group	Identifier/Depository collection	COI 5' (1490–2198)	COI 3' (2195–3014)	16 S	PGD	EF-1a	PERIOD	CAD (54–410)	Specimen data
Stone	<i>pallidipennis</i> complex									Investigación y Capacitación Río Los Amigos (CICRA), Trail #1, near tree 1-225, 12.56694°S 70.10159°W, 261 m, trap LA-ML-05, 9–14.Nov.2013, E. J. Rodríguez & G. J. Steck; female; det. A. L. Norrbom
Anastrepha daciformis Bezzi	<i>daciformis</i>	USNMENT00744444/USNM	KY428336	KY428336	KY428044			KY428566		ARGENTINA: Tucumán, c. 12 km. W of Tucumán, Horco Molle, 26°46'38.2"S 65°19'50.2"W, 28.Jan.2012, S. Ovruski; male; det. A. L. Norrbom
		USNMENT00744557/USNM					KY428473			ARGENTINA: Tucumán, Horco Molle, Parque Sierra San Javier, Antayacu, 26°46'16"S 65°19'28"W, 720 m, trampa 9Q, 5.Oct.2012, J. Altamirano; male; det. A. L. Norrbom
<i>Anastrepha disjuncta</i> Norrbom	<i>panamensis</i>	USNMENT00744139/USNM	KY428266	KY428266				KY428528		PERU: Cusco, Echarate region, Mar–Dec.2011, T. Guevara; paratype female; det. A. L. Norrbom
Anastrepha distincta Greene	<i>fraterculus</i>	USNMENT00671588/USNM	KY428265	KY428265	KY427985	KY428606	KY428436	KY428527		BOLIVIA: Cochabamba, Cono Sur, 17°46'51"S 65°20'28"W, 2038 m, trap 09 in chirimoyo, 23.Feb.2008; female; det. A. L. Norrbom
		05CA009/–							KY428133	GUATEMALA: Ruta, 15.Nov.2001, #39; ALN-2002-68; female; det. A. L. Norrbom
<i>Anastrepha dryas</i> Stone	previously <i>pseudoparallela</i> , here as unknown	PSU-961210-03/–	KY428338		AF152064			AY532499		VENEZUELA: Trujillo, El Helechal, McPhail trap, 9.Sep.1995, K. Katiyar & J. Oroño. (McPheron et al., 1999; Barr et al., 2005)
<i>Anastrepha echaratiensis</i> Norrbom	unknown	USNMENT00744640/USNM	KY428400	KY428400		KY428651		KY428586	KY428212	PERU: Cusco, Echarate region, Palmanayoc – San Augustin, McPhail traps, 2012, T. Guevara; paratype female; det. A. L. Norrbom
Anastrepha edentata Stone	<i>mucronota</i>	USNMENT00212933 (05W017)/USNM	KY428339	KY428339	KY428046				KY428170	DOMINICAN REPUBLIC: Pedernales, Parque Nacional Sierra de Bahoruco, Las Abejas, 18°9.011'N 71°37.342'W, 1310 m, 11.Jul.2004, D. Perez; ALN-2005-34; male; det. A. L. Norrbom
		USNMENT00212133 (TBI-V080416-17)/USNM							KY428567	PUERTO RICO: Mayaguez-Aguadilla; S of Maricao, Rd. 120, km. 14.8, 18.17°N 66.975°W, McPhail trap 120-9, 29. Mar.2007, I. M. Perez & E. O. Rivera; female; det. A. L. Norrbom
<i>Anastrepha ericki</i> Norrbom	<i>mucronota</i> , <i>lanceola</i> clade	USNMENT00104985/USNM	KY428391	KY428391	KY428089	KY428643	KY428498		KY428203	PERU: Cusco, Estación Biológica Villa Carmen, trail 8, 12.90219°S 71.41039°W, 724 m, trap VC-ML-16A, 22–28.Feb.2013, E. J. Rodríguez; paratype female; det. A. L. Norrbom
<i>Anastrepha fenestrella</i> Norrbom & Korytkowski	<i>robusta</i> , <i>robusta</i> clade	USNMENT00671694/USNM	KY428339	KY428339	KY427986	KY428607	KY428437	KY428529	KY428134	PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°12'32"N 79°23'38"W, trap II 4 BM 1, Jun–Aug.2009, E. J. Rodríguez & C. A. Korytkowski; male; det. A. L. Norrbom
<i>Anastrepha flavipennis</i> Greene	unknown	USNMENT00745011/USNM	KY428268	KY428268	KY427987			KY428530		PANAMA: Panamá, Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap I 20 MU2, 28. Aug.2009, E. J. Rodríguez; female; det. C. A. Korytkowski

<i>Anastrepha fraterculus</i> (Wiedemann), Brazil-1 morph	<i>fraterculus</i>	ALN-2006-110/USNM	KY428269	KY428269	KY427988		KY428438			ARGENTINA: Entre Ríos, Concordia, Sep.2006; female; det. A. L. Norrbom
<i>Anastrepha fraterculus</i> (Wiedemann), Peruvian morph	<i>fraterculus</i>	USNMENT00745234/USNM	KY428270	KY428270	KY427989	KY428608	KY428439	KY428531	KY428135	PERU: Huanuco, Huallaga Central, Tingo María, Naranjillo, trampa McPhail, 01.02.01.010, [no date]; female; det. A. L. Norrbom
<i>Anastrepha furcata</i> Lima	<i>robusta, robusta</i> clade	USNMENT00744897/USNM	KY428379	KY428379			KY428486	KY428576	KY428191	PERU: Cusco, Estación Biológica Villa Carmen, trail 7, below 'Orilla antigua', approx. 7-230, 12°53'31"S 71°24'17"W, 541 m, trap VC-ML-06, 4.Feb.2013, E. J. Rodriguez; female; det. A. L. Norrbom
<i>Anastrepha furcata</i> Lima	<i>robusta, robusta</i> clade	USNMENT00671688/MEUP	KY428272	KY428272	KY427990					PANAMA: Coclé, Cerro La Vieja, McPhail trap, 22.Jul.2007, P. Rodriguez; female; det. C. A. Korytkowski
<i>Anastrepha fuscata</i> Norrbom & Korytkowski	<i>robusta, robusta</i> clade	USNMENT00745410/USNM	KY428271	KY428271						PERU: Huánuco, Alto Huallaga, Aucayacu, Anda, McPhail trap 1,1,2,4, 12.Apr.2005, D. Damas SENASA 660-05; female; det. A. L. Norrbom
<i>Anastrepha fuscicauda</i> Norrbom & Korytkowski	<i>schausi</i>	USNMENT00745037/USNM	KY428273	KY428273	KY427991					PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°12'38.8"N 79°22'37.3"W, 814 m, trap II 5 UA2, 28. Aug.2009, E. J. Rodriguez; male; det. C. A. Korytkowski
<i>Anastrepha galbina</i> Stone	<i>mucronota</i>	USNMENT00745019/USNM	KY428274	KY428274	KY427992		KY428440	KY428532		PANAMA: Panamá, Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap I 10 BM2, 21. May.2010, E. J. Rodriguez; female; det. A. L. Norrbom
<i>Anastrepha grandicula</i> Norrbom	<i>grandis</i>	USNMENT00671776/SENASA	KY428275	KY428275						PERU: Junín, Chanchamayo, Pichanaki, Perené, 2009, G. Gonzalez I.; male; det. A. L. Norrbom
<i>Anastrepha grandis</i> (Macquart)	<i>grandis</i>	USNMENT00214416/USNM	KY428276	KY428276	KY427993		KY428441	KY428533		PERU: Cajamarca, Jesús, Luñipucro, 8. Feb.2010, trampa McPhail, E. Gaitán; female; det. A. L. Norrbom
<i>Anastrepha hadracantha</i> Norrbom & Korytkowski	<i>mucronota, megacantha</i> clade	USNMENT00744900/USNM	KY428380	KY428380	KY428079	KY428636	KY428487	KY428577	KY428192	PERU: Madre de Dios, Centro de Investigación y Capacitación Rio Los Amigos (CICRA), Trail #2, 12.56126°S 70.1063°W, 277 m, trap LA-ML-09, 9-13. Nov.2013, E. J. Rodriguez & G. J. Steck; female; det. A. L. Norrbom
<i>Anastrepha hamadryas</i> Stone	unknown	USNMENT00215484 (2004PHK188)/USNM	KY428341	KY428341	KY428048				KY428172	PANAMA: Altos de Pacora, 16.Dec.1997, C. A. Korytkowski, McPhail trap 555; ALN-2002-52; female; det. A. L. Norrbom
<i>Anastrepha hamata</i> (Loew)	<i>dentata</i>	PSU-971210-07/-	KY428342		AF152069			AY532510		MEXICO: Veracruz, 1997, M. Aluja et al. (McPherson et al., 1999; Barr et al., 2005)
<i>Anastrepha haplacantha</i> Norrbom & Korytkowski	<i>mucronota, megacantha</i> clade	USNMENT00744901/USNM	KY428381	KY428381	KY428080		KY428488		KY428193	PERU: Cusco, Estación Biológica Villa Carmen, trail 8, end of flat section, 12.90056°S 71.40921°W, 593 m, trap VC-ML-42, 27.Jul-2.Aug.2013; female; det. A. L. Norrbom
<i>Anastrepha hyperacantha</i> Norrbom & Korytkowski	<i>mucronota, megacantha</i> clade	USNMENT00744577/USNM	KY428402							PERU: Cusco, Estación Biológica Villa Carmen, trail 8 mark 8-1724, 12°54'9"S 71°24'32"W, 702 m, trap VC-ML-15, 27. Nov.2012-20.Jan.2013, A. L. Norrbom, E. J. Rodriguez, G. J. Steck, B.D. Sutton; female; det. A. L. Norrbom
<i>Anastrepha integra</i> (Loew)	<i>mucronota</i>	USNMENT00744578/USNM	KY428343	KY428343	KY428049					PERU: Cusco, Estación Biológica Villa Carmen, trail 5, stream below waterfall,

(continued on next page)

Table 1 (continued)

Taxon	Species group	Identifier/Depository collection	COI 5' (1490–2198)	COI 3' (2195–3014)	16 S	PGD	EF-1a	PERIOD	CAD (54–410)	Specimen data
<i>Anastrepha intermedia</i> Norrbon & Korytkowski	<i>benjamini</i>	USNMENT00671703/USNM	KY428278	KY428278	KY427995	KY428609	KY428442	KY428534	KY428136	12.89244°S 71.41929°W, 543 m, trap VC-ML-19, 27.Nov.2012–21.Jan.2013, G. J. Steck, A.L. Norrbom, E. J. Rodriguez, B.D. Sutton; female; det. A. L. Norrbom PANAMA: Panamá, Parque Nacional Chagres, Cerro Azul, Escuela, 9°8'9.3"N 79°23'29.8"W, 323 m, trap III 17 ML 2, Jun–Aug.2009, E. J. Rodriguez & C. A. Korytkowski; paratype female; det. C. A. Korytkowski
<i>Anastrepha intermedia</i> Norrbon & Korytkowski	<i>benjamini</i>	USNMENT00671701/USNM	KY428277	KY428277	KY427994					PANAMA: Panamá, Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap I 10 UL 1, Jun–Aug.2009, E. J. Rodriguez & C. A. Korytkowski; paratype female; det. C. A. Korytkowski
<i>Anastrepha interrupta</i> Stone	<i>spatulata</i>	USNMENT00671909/USNM	KY428279	KY428279	KY427996					VIRGIN ISLANDS: St. Thomas, Crown Mountain Rd # 33, 18.35374° 64.97238°, fruit fly trap in China berry tree, 29 Mar 2010, C. Cruz STT-70074; female; det. A. L. Norrbom
		USNMENT00875739 (2005PPD020)/USNM							KY428137	USA: FL, Miami Dade Co, Homestead, 25°31' N 80°28' W, ex. <i>Shoepfia chrysophylloides</i> , May.2004, R. Pereira; male; det. A. L. Norrbom
<i>Anastrepha katiyari</i> Norrbon	<i>daciformis</i>	PSU-980326-01/-	KY428344		KY427997			AY532511		VENEZUELA: Zulia, Mara, ex <i>Sideroxylon obtusifolium</i> , 25.Oct.1995, K. Katiyar, Camacho & J. Oroño. (Barr et al., 2005)
<i>Anastrepha korytkowskii</i> Norrbon	<i>mucronota</i>	USNMENT00744872/USNM	KY428387	KY428387	KY428085		KY428494		KY428199	PERU: Cusco, Estación Biológica Villa Carmen, trail 5, 12.89056°S 71.42075°W, 570 m, trap VC-ML-41, 27.Jul–2.Aug.2013; paratype female; det. A. L. Norrbom
<i>Anastrepha lanceola</i> Stone	<i>mucronota, lanceola</i> clade	USNMENT00745038/USNM	KY428280	KY428280	KY427998	KY428610	KY428443		KY428138	PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°12'38.8"N 79°22'37.3"W, 814 m, trap II UT2, 12. Jun.2009, E. J. Rodriguez; female; det. C. A. Korytkowski
<i>Anastrepha latilanceola</i> Norrbon	<i>mucronota, lanceola</i> clade	USNMENT00744904/USNM	KY428395	KY428395	KY428093	KY428646		KY428582	KY428207	PERU: Cusco, Estación Biológica Villa Carmen, trail 8, mark 8–1952, 12°54'8"S 71°24'38"W, 718 m, trap VC-ML-17, 27. Aug–16.Sep.2013; paratype female; det. A. L. Norrbom
<i>Anastrepha leptozona</i> Hendel	<i>leptozona</i>	USNMENT00745231/USNM	KY428281	KY428281	KY427999	KY428611	KY428444	KY428535	KY428139	PERU: Huánuco, Huallaga central, Tingo María, Naranjillo, 1.2.1.10, N. Nolazco; female; det. A. L. Norrbom
<i>Anastrepha levefasciata</i> Norrbon & Korytkowski	<i>raveni</i>	USNMENT00671683/USNM	KY428282	KY428282	KY428000		KY428445	KY428536		PERU: Junín, Chanchamayo, Pichanaki, Villa Santa María, 21.Oct.2008, J. Avalos; paratype female; det. A. L. Norrbom
<i>Anastrepha levefasciata</i> Norrbon & Korytkowski	<i>raveni</i>	USNMENT00213704/USNM	KY428364	KY428364	KY428064					PERU: Huánuco: Tingo María, [no date], G. Egoavil M010, ALN-2004-53; paratype female; det. A. L. Norrbom
<i>Anastrepha limae</i> Stone	<i>pseudoparallela</i>	USNMENT00745036/USNM	KY428284	KY428284						PANAMA: Panamá, Parque Nacional Chagres, Cerro Azul, Escuela, 9°8'9.3"N 79°23'29.8"W, 323 m, trap III 2 UA2, 17.

		V080807-16/-		AF152073			AY532513		Jul.2009, E. J. Rodríguez; female; det. C. A. Korytkowski		
		USNMENT00875735 (05V761)/USNM						KY428141	PANAMA: Capira, 8.Oct.1989, G. Tapia & C. A. Korytkowski. (McPherson et al., 1999; Barr et al., 2005)		
<i>Anastrepha ludens</i> (Loew)	<i>fraterculus</i>	USNMENT00671623/USNM	KY428285	KY428285	KY428002			KY428447	KY428538	KY428142	PANAMA: Cerro Campana, 31.May.2002, C. A. Korytkowski; ALN-2002-50; male; det. A. L. Norrbom
<i>Anastrepha ludens</i> (Loew)	<i>fraterculus</i>	XM186/USNM	KY428286	KY428286	KY428003	KY428613	KY428448	KY428539	KY428143	USA: Texas, Willacy Co., 2 yr old colony, ex grapefruit, emerged 12.Jan.2012, X. Mengual	
<i>Anastrepha macra</i> Stone	<i>fraterculus</i>	USNMENT00212670 (06X792)/USNM	KY428345	KY428345	KY428050					PANAMA: Panamá, Parque Nacional Chagres, Altos de Pacora, 9°15'28"N 79°21'24"W, McPhail trap, 2006, C. A. Korytkowski; ALN-2006-87; female; det. C. A. Korytkowski	
<i>Anastrepha macrura</i> Hendel	<i>daciformis</i>	USNMENT00671157/USNM	KY428287	KY428287	KY428004				KY428540	ECUADOR: Guayas, Isla Puná, 2°55'8.7"S 80°10'24"W, Oct.2007, J.R. Lemly; male; det. A. L. Norrbom	
<i>Anastrepha macrura</i> Hendel	<i>daciformis</i>	USNMENT00671855/USNM	KY428288	KY428288	KY428005		KY428449	KY428541		PERU: Piura, San Lorenzo, Aug.2007, E. Najjar; female; det. A. L. Norrbom	
<i>Anastrepha maculata</i> Norrbom	<i>daciformis</i>	USNMENT00875774 (05V842)/USNM			KY428009					PUERTO RICO: Mona L., 9.Aug.2002, S. Madera & J. Navarro; ALN-2003-34; female; det. A. L. Norrbom	
		USNMENT00671990/USNM	KY428292	KY428292						VIRGIN ISLANDS: St. Thomas, Abatoir, Rd # 30, 18.32034° 64.87834°, multilure trap, 1.Mar.2011, N. Martinez; female; det. A. L. Norrbom	
<i>Anastrepha magna</i> Norrbom	<i>benjamini</i>	USNMENT00744905/USNM	KY428382	KY428382	KY428081	KY428637	KY428489	KY428578	KY428194	PERU: Madre de Dios, Centro de Investigación y Capacitación Rio Los Amigos (CICRA), Trail #2, 12.56126°S 70.1063°W, 277 m, trap LA-ML-09, 9-13. Nov.2013, E. J. Rodríguez & G. J. Steck; female; det. A. L. Norrbom	
<i>Anastrepha magna</i> Norrbom	<i>benjamini</i>	USNMENT00671954/SENASA	KY428289	KY428289	KY428006				KY428542	PERU: Junín, Chanchamayo, Perené, Kivinaki, trap 1,5,9004, 3.Nov.2008, G. González; female; det. A. L. Norrbom	
<i>Anastrepha manihoti</i> Lima	<i>spatulata</i>	USNMENT00654067/USNM	KY428290	KY428290	KY428007	KY428614	KY428450	KY428543	KY428144	BOLIVIA: Cochabamba, Carrasco, Tres Pozas, McPhail trap TSS-2 en <i>Carica papaya</i> , 25.Feb.2005, S. Seballos; female; det. A. L. Norrbom	
<i>Anastrepha manihoti</i> Lima	<i>spatulata</i>	USNMENT00745044/USNM	KY428291	KY428291	KY428008		KY428451	KY428544		PANAMA: Panamá, Parque Nacional Chagres, Cerro Azul, Escuela, 9°8'9.3"N 79°23'29.8"W, 323 m, trap III 17 UA1, 28. Aug.2009, E. J. Rodríguez; female; det. C. A. Korytkowski	
<i>Anastrepha manizaliensis</i> Norrbom & Korytkowski	<i>fraterculus</i>	USNMENT00213567/USNM	KY428293	KY428293	KY428010	KY428615	KY428452	KY428545	KY428145	COLOMBIA: Caldas, Manizales, 5°3'17"N 75°29'37"W, emerged 1.Oct.2003, reared ex fruit of cedro negro, <i>Juglans neotropica</i> Diels, ICA; paratype female; det. A. L. Norrbom	
<i>Anastrepha melanoptera</i> Norrbom	<i>dentata</i>	USNMENT00744019/USNM	KY428247	KY428247	KY427969		KY428423	KY428515	KY428120	PERU: Cusco, Echarate region, Mar-Dec.2011, T. Guevara; paratype female; det. A. L. Norrbom	

Table 1 (continued)

Taxon	Species group	Identifier/Depository collection	COI 5' (1490–2198)	COI 3' (2195–3014)	16 S	PGD	EF-1a	PERIOD	CAD (54–410)	Specimen data
Anastrepha minuta Stone	<i>mucronota</i>	USNMENT00875778 (2004PHK033)/USNM							KY428146	GUATEMALA: Ruta, 8.Nov.2001, J. López #35; ALN-2002-63; female; det. A. L. Norrbom
		USNMENT00671712/USNM	KY428294	KY428294					KY428546	PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°12'32"N 79°23'38"W, trap II UP 2, Jun–Aug.2009, E. J. Rodriguez & C. A. Korytkowski; female; det. A. L. Norrbom
Anastrepha montei Lima	<i>spatulata</i>	USNMENT00744135 /USNM	KY428295	KY428295			KY428453		KY428147	PERU: Cusco, Echarate region, Mar–Dec.2011, T. Guevara; female; det. A. L. Norrbom
		V080807-18/-			AF152076				AY532516	VENEZUELA: Trujillo, El Helechal, McPhail trap baited with Staley, 15.Apr.1996, K. Katiyar & J. Oroño. (McPheron et al., 1999; Barr et al., 2005)
<i>Anastrepha nigra</i> Norrbom & Korytkowski	<i>robusta, nigra</i> clade	USNMENT00875770 (2004PHK191)/USNM	KY428346	KY428346	KY428051					PANAMA: Altos de Pacora, 28.Jul.2000, C. A. Korytkowski, McPhail trap 567; ALN-2002-43; female; det. C. A. Korytkowski
Anastrepha nigrifascia Stone	<i>robusta, robusta</i> clade	V080807-20/-			AF152077				AY532517	BAHAMAS: Abaco I., Bahama Star Grove, 9. Jun.1994. (McPheron et al., 1999; Barr et al., 2005)
		USNMENT00875734 (06X662)/USNM	KY428347							USA: Florida, Monroe Co., Big Pine Key to Key West, Apr–Jun.1972, frozen live. Vial #06-560, code 97006. Received from McPheron Lab, Jun.2006, 06LOT316
<i>Anastrepha nigripalpis</i> Hendel	previously unknown, here placed in <i>pseudoparallela</i>	USNMENT00744908/USNM	KY428383	KY428383	KY428082	KY428638	KY428490	KY428579	KY428195	PERU: Cusco, Estación Biológica Villa Carmen, garden, 12.5342°S 71.241°W, 534 m, emerged 15.Mar.2013, reared from fruit of <i>Passiflora venusta</i> Vasquez & Delanoy (VC-12C) collected Feb.2013, E. J. Rodriguez; female; det. A. L. Norrbom
<i>Anastrepha nigrivittata</i> Norrbom & Korytkowski	<i>robusta, lambda</i> clade	USNMENT00744910/USNM	KY428384	KY428384	KY428083	KY428639	KY428491	KY428580	KY428196	PERU: Madre de Dios, Estación Biológica Villa Carmen, trail 13, above junction with trail 1, 12.86922°S 71.42628°W, 681 m., trap VC-ML-35, 23.Feb–1.Mar.2013, E. J. Rodriguez; female; det. E. J. Rodriguez
<i>Anastrepha nolazcoae</i> Norrbom & Korytkowski	<i>mucronota</i>	USNMENT00671971/USNM	KY428297	KY428297	KY428013	KY428616	KY428454	KY428547	KY428148	PERU: Huánuco, Tingo Maria, La Chancadora, 9°11'2''S 75°57'34''W, 651 m, emerged 19.May–10.Jun.2010, reared ex fruit of sapote, <i>Matisia cordata</i> , collected 21.Apr.2010, N. Nolazco & A.L. Norrbom; paratype female; det. A. L. Norrbom
<i>Anastrepha normalis</i> Norrbom	<i>serpentina</i>	PSU-2-8-1995 TNE9 (V080807-30)/-	KY428348		AF152052				AY532518	VENEZUELA: Marcellol, 7.May.1993, K. Katiyar. (McPheron et al., 1999, as "sp. nr. <i>anomala</i> ") (Barr et al., 2005)
<i>Anastrepha obliqua</i> (Macquart)	<i>fraterculus</i>	USNMENT00745977/USNM	KY428301	KY428301	KY428017	KY428619	KY428458	KY428551	KY428152	VENEZUELA: Lara, Tarabana, on <i>Syzygium jambos</i> , 30.Jun.2011, L. Sorondo; female; det. A. L. Norrbom
<i>Anastrepha obliqua</i> (Macquart)	<i>fraterculus</i>	USNMENT00745998/USNM	KY428302	KY428302	KY428018	KY428620	KY428459	KY428552	KY428153	EL SALVADOR: La Libertad, San Juan Opico, Finca Pipil, 13°56.245'N 89°19.443'W, McPhail trap, 2004, Ministerio de Agricultura y Ganadaria; female; det. A. L. Norrbom
<i>Anastrepha obliqua</i>	<i>fraterculus</i>	USNMENT00745953/USNM	KY428300	KY428300	KY428016	KY428618	KY428457	KY428550	KY428151	VENEZUELA: Lara, Tarabana, on <i>Syzygium</i>

Table 1 (continued)

Taxon	Species group	Identifier/Depository collection	COI 5' (1490–2198)	COI 3' (2195–3014)	16 S	PGD	EF-1a	PERIOD	CAD (54–410)	Specimen data
<i>Anastrepha pulchra</i> Stone	<i>serpentina</i>	USNMENT00744912/USNM	KY428393	KY428393	KY428091				KY428205	PERU: Cusco, Estación Biológica Villa Carmen, trail 8, mark 8-1877, 12°54'8"S 71°24'36"W, 721 m, trap VC-ML-16, 1. Mar–12.Apr.2013, M. Choque; female; det. A. L. Norrbom
<i>Anastrepha punctata</i> Hendel	<i>punctata</i>	USNMENT00744552/USNM	KY428356	KY428356	KY428058				KY428568	ARGENTINA: Tucumán, Horco Molle, Parque Sierra San Javier, Antayacu, 26°46'16"S 65°19'28"W, 720 m, trampa 2I, 5.Sep.2012, J. Altamirano; female; det. A. L. Norrbom
		USNMENT00744555/USNM					KY428475			ARGENTINA: Tucumán, Horco Molle, Parque Sierra San Javier, Antayacu, 26°46'16"S 65°19'28"W, 720 m, trampa 2I, 5.Sep.2012, J. Altamirano; female; det. A. L. Norrbom
<i>Anastrepha punensis</i> Tigero	<i>daciformis</i>	USNMENT00671853/USNM	KY428308	KY428308	KY428022	KY428623	KY428463	KY428555		PERU: Piura, San Lorenzo, Aug.2007, E. Najjar; female; det. A. L. Norrbom
<i>Anastrepha rafaeli</i> Norrbom & Korytkowski	<i>robusta, speciosa</i> clade	USNMENT00744913/USNM	KY428394	KY428394	KY428092	KY428645	KY428500		KY428206	PERU: Madre de Dios, Centro de Investigación y Capacitación Río Los Amigos (CICRA), Trail #22, near intersection trails 22 & 2, 12.55691°S 70.1025°W, 277 m, trap LA-ML-29, 9–14. Nov.2013, E. J. Rodríguez & G. J. Steck; female; det. A. L. Norrbom
<i>Anastrepha ramosa</i> Stone	<i>ramosa</i>	USNMENT00745954/USNM	KY428309	KY428309	KY428023		KY428464	KY428557		PANAMA: Colón, Fort Sherman, 9°21'54"N 79°57'31"W, ex <i>Symphonia globulifera</i> , C. A. Korytkowski; female; det. C. A. Korytkowski
<i>Anastrepha raveni</i> Norrbom & Korytkowski	<i>raveni</i>	USNMENT00875764 (05v631)/ USNM	KY428357	KY428357	KY428059					PERU: Tingo Maria area, G. Egoavil M001; ALN-2004-51; male; det. A. L. Norrbom
<i>Anastrepha rheediae</i> Stone	unknown	USNMENT00875761 (06X779)/ USNM	KY428310	KY428310	KY428024				KY428158	PANAMA: Panamá, Altos de Pacora, McPhail Trap 536, 15.June.2003, C.A. Korytkowski; ALN-2006-48; female; det. A. L. Norrbom
<i>Anastrepha robusta</i> Greene	<i>robusta, robusta</i> clade	USNMENT00875758 (2004PHK052)/USNM	KY428358	KY428358	KY428060				KY428178	GUATEMALA: Ruta, 20.Sep.2001, Lopez #43; ALN-2002-61; female; det. A. L. Norrbom
<i>Anastrepha robynae</i> Norrbom	previously <i>mucronota</i> , here as unknown	USNMENT00744873/USNM	KY428388	KY428388	KY428086	KY428640	KY428495		KY428200	PERU: Madre de Dios, Estación Biológica Villa Carmen, trail 13, at trail fork below junction with trail 1, 12.8701°S 71.42641°W, 661 m, trap VC-ML-34, 23. Feb–1.Mar.2013, E. J. Rodríguez; paratype female; det. A. L. Norrbom
<i>Anastrepha robynae</i> Norrbom	previously <i>mucronota</i> , here as unknown	USNMENT00213408/USNM	KY428363	KY428363	KY428063					PERU: Huánuco, Tingo María, G. Egoavil; ALN-2004-52; paratype female; det. A. L. Norrbom
<i>Anastrepha rosilloi</i> Blanchard	previously unknown, here placed in <i>pseudoparallela</i>	USNMENT00744502/USNM	KY428359	KY428359			KY428476	KY428569		ARGENTINA: Tucumán, Horco Molle, Parque Sierra San Javier, Antayacu, 26°46'16"S 65°19'28"W, 720 m, trampa, 5. Sep.2012, J. Altamirano; female; det. A. L. Norrbom
<i>Anastrepha sagittata</i> (Stone)	<i>dentata</i>	USNMENT00875729 (05CA016)/ USNM	KY428360	KY428360	KY428061					GUATEMALA: Peña Plata, 13.Jun.2001, Lopez #23; ALN-2002-60; female, det. A.

Anastrepha serpentina (Wiedemann)	<i>serpentina</i>	USNMENT00745219/USNM 6-20-1994/- USNMENT00875728 (05V059)/USNM	KY428311	KY428311	KY428025		KY428465			L. Norrbom PERU: Huánuco, Huallaga central, Tingo María, Naranjillo, 01.02.01.010, N. Nolzco; female; det. A. L. Norrbom VENEZUELA: Aragua, Maracay, ex. <i>Manilkara zapota</i> , 1988, G. J. Steck. (Barr et al., 2005)
<i>Anastrepha shannoni</i> Stone	<i>grandis</i>	USNMENT00744020/USNM	KY428312	KY428312	KY428026		KY428466		KY428159	BRAZIL: Espírito Santo, Linhares, Atlantic rain forest, 28.Jan-3.Feb.2003, K. Uramoto; female; det. K. Uramoto PERU: Cusco, Echarate region, Mar-Dec.2011, T. Guevara; male; det. A. L. Norrbom
<i>Anastrepha similis</i> Greene	<i>mucronota</i>	USNMENT00671979/USNM	KY428313	KY428313	KY428027					PANAMA: Panamá, Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, 11.Mar.2010, E. J. Rodriguez; female; det. E. J. Rodriguez
<i>Anastrepha sororcula</i> Zucchi	<i>fraterculus</i>	USNMENT00671589/USNM	KY428314	KY428314	KY428028		KY428467	AY532526		BOLIVIA: Cochabamba, Cono Sur, Mizque, 17°56'38"S 65°21'49"W, 2044 m, McPhail trap 09 in guayaba, 29.Feb.2008, Rojas; female; det. A. L. Norrbom
<i>Anastrepha</i> sp.1 [dentata group]	<i>dentata</i>	USNMENT00745051/USNM	KY428296	KY428296	KY428012					PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°12'38.8"N 79°22'37.3"W, 814 m., trap II 19 UP2, 23. Apr.2010, E. J. Rodriguez; female; det. E. J. Rodriguez
<i>Anastrepha</i> sp.2 [dentata group]	<i>dentata</i>	USNMENT00745042/USNM	KY428307	KY428307	KY428021	KY428622	KY428462		KY428157	PANAMA: Panamá, Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap I 22 UU1, 11. Feb.2010, E. J. Rodriguez; male; det. A. L. Norrbom
<i>Anastrepha</i> sp.3 [dentata group]	<i>dentata</i>	USNMENT00744530/USNM	KY428337	KY428337	KY428045		KY428474		KY428169	PERU: Cusco, Estación Biológica Villa Carmen, trail 8, ridge, 8-1877, WPE-22, 12.90217°S 71.41014°W, 729 m, emerged 1-5.Mar.2013, reared ex fruit of <i>Pouteria</i> sp. (VC-07) collected 26.Jan.2013, G. J. Steck, E. J. Rodriguez & A. L. Norrbom; female; det. A. L. Norrbom
<i>Anastrepha</i> sp.4	<i>mucronota</i>	USNMENT00745052/USNM	KY428255	KY428255	KY427976		KY428427			PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°12'38.8"N 79°22'37.3"W, 814 m, trap II 19 ML1, 26. Mar.2010, E. J. Rodriguez; female; det. C. A. Korytkowski
<i>Anastrepha</i> sp. [BOL-02]	unknown	USNMENT00744992/USNM	KY428374	KY428374	KY428074	KY428633	KY428481		KY428186	BOLIVIA: Santa Cruz, Ichilo Prov., 4-6 km. SSE of Buena Vista, Hotel Flora y Fauna, 2nd river trail, 17.502°S 63.6483°W, 385 m, trap FF-ML-19, 7-9.Mar.2014, G. J. Steck, B. D. Sutton, A. L. Norrbom & A. Saravia; female; det. A. L. Norrbom
<i>Anastrepha</i> sp. [nr. <i>limae</i> Stone & <i>anduzei</i> Stone]	<i>pseudoparallela</i>	USNMENT00212023/USNM	KY428283	KY428283	KY428001	KY428612	KY428446	KY428537	KY428140	GUATEMALA: Baja Verapaz, Finca Santa Rosa, Pantin - Salama Road, 15.24895°N 90.28367°W, 1665 m, emerged 21-31. Dec.2007, reared ex fruit of <i>Passiflora sexflora</i> (07G43) collected 20.Nov.2007, B. D. Sutton, G.J. Steck, A.L. Norrbom, J. Monzón; female; det. A. L. Norrbom
<i>Anastrepha</i> sp. [nr. <i>margarita</i> Caraballo]	<i>panamensis</i>	USNMENT00744115/USNM	KY428299	KY428299	KY428015		KY428456	KY428549	KY428150	PERU: Cusco, Echarate region, Mar-Dec.2011, T. Guevara; female; det. A. L. Norrbom

Table 1 (continued)

Taxon	Species group	Identifier/Depository collection	COI 5' (1490–2198)	COI 3' (2195–3014)	16 S	PGD	EF-1a	PERIOD	CAD (54–410)	Specimen data
<i>Anastrepha</i> sp. [nr. <i>nascimentoi</i> Zucchi]	<i>spatulata</i>	USNMENT00875747 (2004PHK055)/USNM	KY428365	KY428365	KY428065				KY428181	GUATEMALA: San Bartolome, Milpas Altas, km. 4817, 30.Jan.2002; ALN-2002-75; female; det. A. L. Norrbom
<i>Anastrepha</i> sp. [nr. <i>parishi</i> Stone]	<i>pseudoparallela</i>	USNMENT00671595/USNM	KY428298	KY428298	KY428617	KY428617	KY428455	KY428548	KY428149	BOLIVIA: Cochabamba, Chimore, 16°51'14"S 65°6'31"W, 196 m, McPhail trap 10 in naranja, 11.Apr.2008, Salinas; female; det. A. L. Norrbom
<i>Anastrepha</i> sp. [nr. <i>pittieri</i> Caraballo]	<i>robusta, robusta</i> clade	USNMENT00744917/USNM	KY428392	KY428392	KY428090	KY428644	KY428499	KY428581	KY428204	PERU: Madre de Dios, Estación Biológica Villa Carmen, trail 13, at trail fork below junction with trail 1, 12.8701°S 71.42641°W, 661 m, trap VC-ML-34, 23. Feb.–1.Mar.2013, E. J. Rodriguez; female; det. A. L. Norrbom
<i>Anastrepha</i> sp. [nr. <i>submunda</i> Lima]	<i>mucronota</i>	06X801/USNM	KY428366	KY428366	KY428066					PANAMA: Altos de Pacora, trap 552b, Jan.2000, C. A. Korytkowski; ALN-2006-43; male; det. C. A. Korytkowski
<i>Anastrepha</i> sp. [nr. <i>tumbalai</i> Tigrero]	<i>mucronota, lanceola</i> clade	USNMENT00744137/USNM	KY428320	KY428320	KY428034	KY428627	KY428471	KY428562	KY428164	PERU: Cusco, Echarate region, Mar–Dec.2011, T. Guevara; female; det. A. L. Norrbom
<i>Anastrepha</i> sp. [nr. <i>willei</i> Korytkowski]	<i>mucronota</i>	USNMENT00744916/USNM	KY428397	KY428397	KY428095	KY428648	KY428502	KY428584	KY428209	PERU: Cusco, Estación Biológica Villa Carmen, trail 5, stream after large pasture, 12°53'37"S 71°25'9"W, 568 m, trap VC-ML-10, 26.Jan–1.Feb.2013, E. J. Rodriguez; female; det. A. L. Norrbom
<i>Anastrepha</i> sp. [Peru-45]	<i>mucronota, lanceola</i> clade	USNMENT00744620/USNM	KY428404			KY428652		KY428587	KY428214	PERU: Cusco, Echarate region, Palmanayoc – San Augustin, McPhail traps, 2012, T. Guevara; female; det. A. L. Norrbom
<i>Anastrepha</i> sp. [Peru-58]	unknown	USNMENT00744835	KY428406	KY428406	KY428101				KY428216	PERU: Cusco, Estación Biológica Villa Carmen, trail 5, beyond stream with waterfall, 12.89168°S 71.41985°W, 560 m, trap VC-ML-40A, 27.Aug–16.Sep.2013; male; det. A. L. Norrbom
<i>Anastrepha</i> sp. [Peru-59]	<i>pseudoparallela</i>	USNMENT00744879/USNM	KY428407	KY428407					KY428217	PERU: Madre de Dios, Estación Biológica Villa Carmen, trail 13, near high point, 12.86846°S 71.42572°W, 698 m, trap VC-ML-39, 23.Feb–1.Mar.2013, E. J. Rodriguez; female; det. A. L. Norrbom
<i>Anastrepha spatulata</i> Stone	<i>spatulata</i>	USNMENT00745030/USNM	KY428304	KY428304						PANAMA: Panamá: Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap I 14 UU2, 08. Apr.2010, E. J. Rodriguez; male; det. C. A. Korytkowski
		2-8-95-15/–			AF152087			AY532527		TOBAGO: Area II (St. David or St. George Par.), trap in mango, 27.Jun.1989. (McPheron et al., 1999; Barr et al., 2005)
		USNMENT00213003 (05V999)/USNM							KY428155	EL SALVADOR: La Libertad, San Juan Opico, Finca Pipil, 13°56.245'N 89°19.443'W, McPhail trap, 2004, MAG; female; det. A. L. Norrbom
<i>Anastrepha speciosa</i> Stone	<i>robusta, speciosa</i> clade	USNMENT00745024/USNM	KY428315	KY428315						PANAMA: Panamá, Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap I 15 UA2, 25. Feb.2010, E. J. Rodriguez; female; det. E. J. Rodriguez

		USNMENT00875756 (05v569)/ USNM							KY428160	PANAMA: Altos de Pacora, 12.Apr.2002, C. A. Korytkowski; ALN-2002-37; female; det. C. A. Korytkowski
		USNMENT00875753 (07Y643)/ USNM							KY428029	PANAMA: Panamá, Altos de Pacora, McPhail Trap, 2005. C.A. Korytkowski; ALN-2006-13A; female; det. C. A. Korytkowski
		X061006-021/ – (individual destroyed, but additional specimens in sample)							KY428558	PANAMA: Altos de Pacora, 2002, C. A. Korytkowski, McPhail trap; ALN-2006-14B; det. C. A. Korytkowski
<i>Anastrepha striata</i> Schiner	<i>striata</i>	USNMENT00744040/USNM	KY428316	KY428316	KY428030	KY428624	KY428468	KY428559	KY428161	PERU: Cusco, Carretera Manu, Cosñipata Valley, Cock of Rock Lodge, reared ex fruit of <i>Psidium guajava</i> , 6.Dec.2011, Norrbom, Steck, Sutton & Nolazco; female; det. A. L. Norrbom
<i>Anastrepha subramosa</i> Stone	<i>ramosa</i>	USNMENT00212685/USNM	KY428367	KY428367	KY428067					PANAMA: Altos de Pacora, 15.Sep.1995, C. A. Korytkowski, McPhail trap 573a; female; det. C. A. Korytkowski
<i>Anastrepha superflua</i> Stone	<i>benjamini</i>	USNMENT00745053/USNM	KY428317	KY428317	KY428031	KY428625	KY428469	KY428560	KY428162	PANAMA: Panamá, Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap 1 22 ML2, 22. Oct.2009, E. J. Rodriguez; female; det. E. J. Rodriguez
<i>Anastrepha suspensa</i> (Loew)	<i>fraterculus</i>	USNMENT00745987/USNM	KY428318	KY428318	KY428032	KY428626	KY428470	KY428561	KY428163	USA: Florida, Gainesville, DPI Lab colony, 14.Feb 2006; female
<i>Anastrepha sylvicola</i> Knab	unknown	PSU-02-09-1995 TNE3/-	KY428368		KY428068			AY532530		VENEZUELA: Falcón, Boca Tocuyo, 1995 (Barr et al., 2005)
<i>Anastrepha tecta</i> Zucchi	<i>spatulata</i>	USNMENT00671957/USNM	KY428319	KY428319	KY428033					PERU: Cajamarca, Alto Jequetepeque, Gallito Ciego, Tembladera, McPhail trap 6.1.1.3, 6.Jan.2010, C. Olivera; female; det. A. L. Norrbom
<i>Anastrepha tehucana</i> Norrbom	<i>tripunctata</i>	USNMENT00671835/USNM	KY428408	KY428408			KY428505	KY428588		MEXICO: Puebla, Mpio. Tehuacán, Ejido del Santiago Tula, 18°29'26"N 97°22'2"W, reared ex fruit of <i>Euphorbia tehucana</i> , 21. Nov.2008, E. Acosta; paratype female; det. A. L. Norrbom
<i>Anastrepha tumida</i> Stone	unknown	USNMENT00875727 (06X514) / USNM							KY428094	PANAMA: Chica, McPhail trap 628, 2. Jul.2004, C. A. Korytkowski; ALN-2006-32; female; det. C. A. Korytkowski
		USNMENT00744915/USNM	KY428396	KY428396		KY428647	KY428501		KY428208	PERU: Cusco, Estación Biológica Villa Carmen, trail 8 between marks 8-1799 & 8-1877, 12.9024°S 71.40976°W, 721 m, trap VC-ML-48, 27.Aug-16.Sep.2013; female; det. A. L. Norrbom
		V090812-015/APHIS							KY428583	PANAMA: Panama, Canal Zone, Gamboa, 01.Jun.2009; 0090720-001-E-001-S01
<i>Anastrepha woodleyi</i> Norrbom & Korytkowski	<i>serpentina</i>	USNMENT00104215/USNM	KY428321	KY428321		KY428628		KY428563		BOLIVIA: Santa Cruz, Andrés Ibañez, Potrerillos del Guenda, 17°40'S 63°27'W, 400 m, at light, 23.Oct.2007, N. E. Woodley; holotype female; det. A. L. Norrbom
		USNMENT00104216 (TBI- V080625-20)/ USNM							KY428035	BOLIVIA: Santa Cruz, Andrés Ibañez, Potrerillos del Guenda, 17°40'S 63°27'W, 400 m, at light, 26.Oct.2007, N. E. Woodley; paratype male; det. A. L. Norrbom
<i>Anastrepha zenildae</i> Zucchi	<i>fraterculus</i>	990927-10/- USNMENT00744581/USNM							KY428069	BRAZIL: Rio Grande Do Norte, Mossoro, ex goiaba, 04.Dec.1999
			KY428369	KY428369						PERU: Cusco, Estación Biológica Villa

(continued on next page)

Table 1 (continued)

Taxon	Species group	Identifier/Depository collection	COI 5' (1490–2198)	COI 3' (2195–3014)	16 S	PGD	EF-1a	PERIOD	CAD (54–410)	Specimen data
<i>Anastrepha zetekii</i> Greene	panamensis	USNMENT00745014/USNM	KY428322	KY428322	KY428036	KY428629	KY428472	KY428564	KY428165	Carmen, garden area, 12°53'42"S 71°24'10"W, 534 m, trap VC-ML-21 in guava tree on canal, 6–11.Jan.2013, T. Förster; female; det. A. L. Norrbom (identification tentative)
<i>Anastrepha zetekii</i> Greene	panamensis	USNMENT00212694/USNM	KY428370	KY428370	KY428070				KY428182	PANAMA: Panamá, Parque Nacional Chagres, Doña Julia, 9°13'42.3"N 79°21'54.2"W, 747 m, trap II 13 UL2, 08. Apr.2010, E. J. Rodriguez; male; det. A. L. Norrbom
<i>Anastrepha zuelaniae</i> Stone	fraterculus	USNMENT00214987 (05V807)/USNM	KY428371	KY428371	KY428071			KY428573	KY428183	MEXICO: Veracruz, Martínez de la Torre, 19°58'N 96°47'W, McPhail trap 2 in toronja orchard, 17.Jun.2000, M. Aluja; ALN-2002-90; female; det. A. L. Norrbom
<i>Bactrocera distincta</i> (Malloch)		FBA031453/USNM	KY428230	KY428230	KY427953	KY428591	KY428411	KY428508	KY428110	FIJI: Kaduva Island, Kaduva Prov., Namalata, 19°02'318"S 178°11'140"E 120 m, FJ-60D Malaise, 15–28.Jul.2004, M. Irwin, E. Schlinger, M. Tokoka'a; male; det. A. L. Norrbom
<i>Blepharoneura perkinsi</i> Condon & Norrbom		Bpup208/BARC	KY428218	KY428218	KY427943	KY428589	KY428409	KY428506	KY428102	PERU: Madre de Dios, CICRA Los Amigos Biological Station, 17.Oct.2008; pupa; ex. <i>Gurania spinulosa</i> flower, 190-29; male; det. M. Lewis
<i>Ceratitis capitata</i> (Wiedemann)		USNMENT00745899/USNM	KY428231	KY428231	KY427954		KC177514			BOLIVIA: Tarija, Bermejo, 22°39'S 64°22'W, McPhail trap, 14.Aug.2005, R. Urban; female; det. A. L. Norrbom (Wiegmann et al., 2011)
<i>Campiglossa genalis</i> (Thomson)		05V124/USNM	KY428228	KY428228	KY427951		KT594858	KT594818	EF126974	U.S.A.: Hawaii, Oahu. (Leblanc et al., 2015)
<i>Dioxyyna picciola</i> (Bigot)		2004PHK007/USNM	KY428227	KY428227	KY427950				KY428107	KENYA: Msambweni coast, 31.Dec.2000, R. Copeland (Barr and Wiegmann, 2009)
<i>Euphranta lemniscata</i> (Enderlein)		FBA023720 (XM197)/USNM	KY428246	KY428246		KY428596	KY428422	KY428514	KY428119	USA: California, Plumas Co., 1.6 mi NW of Hwy. 70 on Willow Creek Rd., 39°49'11"N, 120°32'49"W, 5100ft, reared from <i>Crepis occidentalis</i> Nutt., 29.Jun.2004, P. H. Kerr; female; det. P. H. Kerr
<i>Hexachaeta amabilis</i> (Loew)	amabilis	PSU-05-21-1994 TNE 8/-	KY428225		U39382					USA: Florida, Miami-Dade Co., Kendall, USDA Subtropical Agricultural Research Center, 25°38'35.9"N 80°17'38.9"W, 19. May.2004, P. H. Kerr
<i>Hexachaeta eximia</i> (Wiedemann)	eximia	USNMENT00745888/USNM	KY428239	KY428239	KY427962		KY428417		KY428115	FIJI: Viti Levu Island, Vuda Prov., Koroyanitu N. H. P. Savuione Trail, 17°40'S 177°33'E 450 m, FJ-1 Malaise, 12–19. Oct.2002, M. Irwin, E. Schlinger, M. Tokoka'a; female; det. A. L. Norrbom
										GUATEMALA: Escuintla: Palín area, McPhail trap, 1992-1993, J. López, (McPheron et al., 1999)
										BRAZIL: Rio de Janeiro, Cambuci, McPhail trap, 22.Sep.2004, E. L. Aguiar-Menezes;

<i>Hexachaeta fallax</i> Lima	<i>socialis</i>	PSU-2-8-95-16/-	KY428224	AF152089	AY532534						male; det. by A. L. Norrbom MEXICO: Nuevo Leon, Santiago, 24. Feb.1994, Esau (McPheron et al., 1999; Barr et al., 2005)
<i>Hexachaeta seabrai</i> Lima	<i>eximia</i>	USNMENT00213020 (05V985)/ USNM	KY428223	KY428223	KY427948						MEXICO: Veracruz, Apazapan, 19°19'N 96°43'W, McPhail trap M-6, 9Jun.2004, M. Aluja; ALN-2005-12; female; det. by A. L. Norrbom
<i>Hexachaeta</i> sp.1 [<i>amabilis</i> group]	<i>amabilis</i>	USNMENT00671715/USNM	KY428238	KY428238	KY427961					KY428416	PANAMA: Darién, Santa Fe, 8°39'29"N 78°9'5"W, McPhail trap, 16.Aug.2009, A. L. Norrbom & C. A. Korytkowski; female; det. A. L. Norrbom
<i>Hexachaeta</i> sp.2 [<i>amabilis</i> group]	<i>amabilis</i>	USNMENT00213511/USNM	KY428240	KY428240	KY427963						BOLIVIA: Santa Cruz, Ichilo Prov., 4-6 km. SSE of Buena Vista, Hotel Flora y Fauna, 17°29.95'S 63°33.15'W, 400-500 m, 11. Nov.2003, N. E. Woodley; male; det. A. L. Norrbom
<i>Hexachaeta</i> sp. [<i>socialis</i> group]	<i>socialis</i>	USNMENT00745889/USNM	KY428241	KY428241	KY427964						PANAMA: Panamá, Parque Nacional Chagres, Altos de Pacora, 9°15'28"N 79°21'24"W, McPhail traps, 1997, C. A. Korytkowski; male; det. A. L. Norrbom
<i>Parastenopa limata</i> (Coquillett)		USNMENT00671659/USNM	KY428233	KY428233	KY427956	KY428593	KY428413	KY428509	KY428112		USA: New Jersey, Cape May Co., Town Bank, 38°59'28.5"N 74°57'24.1"W, emerged 23 Aug 2009 reared ex fruit of <i>Ilex</i> sp. collected 24 Jul 2009, A. L. Norrbom; male; det. A. L. Norrbom
<i>Pseudophorellia diffusa</i> Norrbom		USNMENT00671704/USNM	KY428236	KY428236	KY427959					KY428114	PANAMA: Panamá, Parque Nacional Chagres, Cerro Jefe, 9°13'36"N 79°21'54"W, trap I UT 2, Jun-Aug.2009, E. J. Rodriguez & C. A. Korytkowski; female; det. A. L. Norrbom
<i>Pseudophorellia</i> <i>fenestrata</i> Norrbom		USNMENT00213746 (05V844)/ USNM	KY428222	KY428222	KY427947						PANAMA: Panamá, Parque Nacional Chagres, Altos de Pacora, 9°15'28"N 79°21'24"W, Desvio, McPhail trap 530, 17. Feb.1995, C. A. Korytkowski; ALN-2003- 36; paratype female; det. A. L. Norrbom
<i>Rhagoletis basiola</i> (Osten Sacken)		USNMENT00671816/USNM	KY428232	KY428232	KY427955	KY428592	KY428412			KY428111	USA: Massachusetts, Martha's Vineyard, Menemsha, 41°21'5"N 70°45'46"W, emerged 1-11.Jan.2010, ex fruit of <i>Rosa</i> sp. collected 13.Aug.2008, A. L. Norrbom; female; det. A. L. Norrbom
<i>Strauzia longitudinalis</i> (Loew)		USNMENT00745217/USNM	KY428235	KY428235	KY427958					KY428415	USA: Virginia, Arlington, W&OD bike trail, btw. Ohio Street & Patrick Henry Drive, 38°52'51.7"N 77°8'35"W, emerged 6-10. Apr.2010, reared ex 2009 stems prob. of <i>Helianthus decapetalus</i> collected 27. Mar.2010, A. L. Norrbom; female; det. A. L. Norrbom
<i>Tachinisca cyaneiventris</i> Kertész		2004PHK223/CSCA	KY428221	KY428221	KY427946					KY428105	BOLIVIA: La Paz: 50 km N. Caranavi, 15°39.502'S 67°26.57' W, 1100 masl, 17. Apr.2004, P.H. Kerr; male; det. P. H. Kerr
<i>Toxotrypana australis</i> Blanchard										AY532484	ARGENTINA: Tucumán, Burruyacu, Taruca Pampa, Finca San Augustine, McPhail trap in citrus orchard, 14.May.1992. (McPheron et al., 1999)
		USNMENT00744127/USNM	KY428242	KY428242	KY427965					KY428418	BOLIVIA: Tarija, Carapari, 06-04-03, McPhail trap, 2008; female; det. A. L. Norrbom
<i>Toxotrypana curvicauda</i>		USNMENT00671713/USNM	KY428243	KY428243	KY427966	KY428594	KY428419	KY428511	KY428117		PANAMA: Darién, La Moneda, 8°35'38"N

(continued on next page)

Table 1 (continued)

Taxon	Species group	Identifier/Depository collection	COI 5' (1490–2198)	COI 3' (2195–3014)	16 S	PGD	EF-1a	PERIOD	CAD (54–410)	Specimen data
Gerstaecker										78°3'52"W, on papaya fruit, 16.Aug.2009, A. L. Norrbom & C. A. Korytkowski; male; det. A. L. Norrbom
<i>Toxotrypana littoralis</i> Blanchard		USNMENT00744919/USNM	KY428398	KY428398	KY428096	KY428649	KY428503		KY428210	PERU: Cusco, Estación Biológica Villa Carmen, trail 5, 12.89219°S 71.41946°W, 559 m, trap VC-ML-49, 24–30.Apr.2013, M. Choque; female; det. A. L. Norrbom
<i>Toxotrypana littoralis</i> Blanchard		USNMENT00744113/USNM	KY428245	KY428245	KY427968		KY428421			PERU: Cusco, Echarate region, Mar–Dec.2011, T. Guevara; female; det. A. L. Norrbom
		2-8-95-18/–							KY428513	GUATEMALA: El Portal, May.1994; det. H. Y. Han
<i>Toxotrypana littoralis</i> Blanchard		USNMENT00745223/USNM	KY428244	KY428244	KY427967	KY428595	KY428420	KY428512	KY428118	PERU: Huánuco, Tingo Maria, Pozo Azul, 9°11'1" S 75°57'58"W, 721 m, SENASA trap 1.2.2.11, 21.Apr.2010, D. Damas; female; det. A. L. Norrbom
<i>Toxotrypana nigra</i> Blanchard		USNMENT00744925/USNM	KY428399	KY428399	KY428097	KY428650	KY428504	KY428585	KY428211	BOLIVIA: Cochabamba, Aiguile, 18°19'34"S 65°13'43"W, 04-06-03-06, 2080 m, McPhail trap, 22.Jan.2009; female; det. A. L. Norrbom
<i>Xanthaciura insecta</i> (Loew)		USNMENT00875645 (2004PHK005)/USNM				KY427945			KY428104	USA: Florida, Miami-Dade Co., Kendall, USDA Subtropical Agricultural Research Center, 25°38'35.9"N 80°17'38.9"W, 19. May.2004, P. H. Kerr; male; det. P. H. Kerr
		USNMENT00875646 (05V582)/USNM	KY428220	KY428220						USA: Florida, Miami-Dade Co., Kendall, USDA Subtropical Agricultural Research Center, 25°38'35.9"N 80°17'38.9"W, 19. May.2004, P. H. Kerr; male; det. P. H. Kerr
<i>Zonosemata electa</i> (Say)		USNMENT00671936/USNM	KY428234	KY428234	KY427957		KY428414			USA: South Carolina, Cherokee, 35.12687°N 81.77537°W, farm, 24. May.2010, D. Piner; female; det. A. L. Norrbom

specimens are listed in the third column of Table 1 together with the depository institution, and other related codes and numbers are listed in the 'Specimen data' column. For example, sample codes starting with ALN are in this column. Vouchered specimens are deposited in the following institutions as listed in Table 1:

APHIS – Center for Plant Health Science and Technology Mission Laboratory, USDA-APHIS, Edinburg, USA
 BARC – Beltsville Agricultural Research Center, United States Department of Agriculture, Beltsville, USA
 CSCA – California State Collection of Arthropods, California Department of Food and Agriculture, Sacramento, USA
 MEUP – Museo de Entomología, Universidad de Panama, Panama
 MHNJP – Museo de Historia Natural «Javier Prado», Universidad Nacional Mayor de San Marcos, Lima, Peru
 SENASA – Servicio Nacional de Sanidad Agraria, La Molina, Lima, Peru
 USNM – National Museum of Natural History, Smithsonian Institution, Washington D.C., USA

The examined material was collected from a variety of sources in the New World. Specimen data and species group designation for each species are indicated in Table 1, as well as the GenBank® (National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda MD, 20894 USA) accession numbers for all the sequences.

A total of 146 *Anastrepha* species, representing 19 of the 21 species groups as well as five of the six clades of the *robusta* group, were included in the present analysis; this included 17 species without an assigned group or clade. Only the *caudata* species group, the *doryphoros* species group, and the *binodosa* clade of the *robusta* group were not included. In addition, four species of *Toxotrypana* and seven *Hexachaeta* species, representing three of the four species groups, were also analyzed together with 17 outgroup species representing five subfamilies and 15 different genera. *Blepharoneura perkinsi* Condon & Norrbom (Tephritidae: Blepharoneurinae) was used to root the phylogenetic trees.

When possible, the same voucher specimen used in previous works (McPheron et al., 1999; Smith-Caldas et al., 2001; Barr et al., 2005) was used to obtain additional genes for this study. Most specimens used by McPheron et al. (1999) were destroyed during DNA extraction, although new sequences for additional molecular markers were obtained from the DNA template. Additional sequences for some studied taxa were retrieved from GenBank. In order to minimize the amount of missing data in our molecular matrix we used 'composite taxa' consisting of multiple individuals of the same species, but not chimeric taxa of multiple species. At the end, 30 composite terminals have sequences from different specimens of the same species of a total of 177 studied terminals (see Table 1, species in bold). We used composite species to infer the relationships at a higher level, i.e. species groups and genera, and the assumption that each composite must be monophyletic was not violated (Campbell and Lapointe, 2009).

2.2. DNA extraction and sequencing

We used the DNeasy® Blood & Tissue Kit (Qiagen, Valencia, CA, USA) to extract total nucleic acids from one or two legs or occasionally the abdomen of each fly, and the TaKaRa EX Taq kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) for the PCRs. PCRs (25 µl) included 3–4 µl DNA extract, 1 µl of each primer (at 10 pmol/µl), 0.2 µl of TaKaRa EX Taq polymerase (5 U/µl), 2.5 µl 10x EX Taq buffer, 2 µl 200 mM dNTP, and ultra-pure water. PCR amplifications were carried out with a DNA Engine Tetrad® 2 Thermo Cycler (Bio-Rad, Hercules, CA, USA) with the following

'touchdown' program: initial denaturation for 2 min at 92 °C, 12 touchdown cycles from 58 °C to 46 °C (10 s at 92 °C, 10 s at 58–46 °C, 1.5 min at 72 °C), 27 cycles at 10 s at 92 °C, 10 s at 45 °C, 1.5 min at 72 °C, and a final extension for 7 min at 72 °C. Primers for PCR and DNA sequencing are listed in Table 2. Four new primers were designed for *Anastrepha* species to amplify the COI gene, as well as one primer for PGD; all of them listed in Table 2 with the prefix AnCO followed by a number corresponding to the 3'-most base of the primer compared with the genome of *Drosophila yakuba* Burla (Clary and Wolstenholme, 1985), and followed by F or R for forward or reverse primers, respectively. No introns were amplified for any gene.

PCR products were cleaned for sequencing using ExoSAP-IT® (Affymetrix, Santa Clara, CA, USA) or gel purification using the QIAquick PCR Purification Kit® (Qiagen, Valencia, CA, USA). Sequencing reactions were carried out with the Big Dye® Terminator version 3.1 Cycle Sequencing kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) and fractionated on an ABI PRISM® 3100 Genetic Analyzer or ABI 3730xl DNA Analyzer. Contigs were assembled for each gene region with the software package Geneious version R6 (Biomatters, Auckland, New Zealand). Newly generated sequences (this project) are listed in Table 1 with GenBank accession numbers starting with starting with KY42.

2.3. Sequence alignment

The protein-coding genes COI, EF1a, and PGD were aligned manually and no gap was needed. The alignment of CAD was straightforward, but gaps were needed as the sequence of *Anastrepha panamensis* Greene is three nucleotides longer than the rest. In the alignment of PER, we found two gaps: the first one is 12 nucleotides long and is due to the longer sequences of this gene for the outgroup species, and a second gap of 12 nucleotides to accommodate the longer sequence of *Euphranta lemniscata* (Ender-

Table 2

Primers used for amplifying and sequencing the molecular markers. Bold indicates new primers designed for this study.

Gene	Primer's name	Sequence
COI	TY-J-1460	TACAATCTATCGCCTAAACTTCAGCC
	1461-F	TTTACARITTTACCGCTATTRTCAGCCA
	LCO	GGTCAACAATCATAAAGATATTG
	AnCO-1490F	TTYTCAACAAATCATAAAGATATYGG
	HCO	TAAACTTCAGGGTGACCAAAAAATCA
	1751F	GGAGCTCCTGACATAGCATTTCC
	AnCO-1751F	GGAGCACCYGATATAGCATTYCCACG
	AnCO-2188R	GTAAAAATTARRATRTAAACTTCTGG
	CO-2191R	CCCGGTAATAATAATAAACTTC
	2183F	CARCAYTATTYTGATTTTTTGG
	Jerry	CAACATTTATTTGATTTTTTGG
	AnCO-2368R	GTAAAAATATGCYCGTGRCTCTAC
2413R	TCARCTRAAAATTTAATTCCTGT	
2441F	CCTACAGGAATTAATAATTTTGTGATTAGC	
2441R	GCHADTCADCTRAAAATTTTRATNCC	
2797F	CCTCGACGTTATTCAGATTACC	
PAT	TCATTTGCACTAATCTGCCATATTA	
16S	12887F	CCGGTTTGAACCTCAGATCATGT
	1339R	CRCTGTTTAWCAAAAACAT
CAD	54F	GTNGTNTTYCARACNCGNATGGT
	405R	GCNTRTGYTCNCGRTGRAAYTG
EF-1a	40F	GTCGTGATCGGACACGTCGATTCCGG
	EF-46F	TGAGGAATCAAGAAGGAAG
	TEF-4R	GTTGGCGATTGACCAAGGGTGGTTC
	53R	GGAACTTCAAGCAATGTGAGC
PGD	AnCO-2.1F	GAYATGCAATTGATTGTYGAAGC
	2.5R	ATRAACCNCCRCGCCACAT
PERIOD	2612F	ATTTCATGGGAAGGAGATGCC
	2612F deg	ATTCWTGGGARGRGATGCC
	3105R	AABGACATGGGTTGGTACATC

Table 3

Selected evolutionary model to best fit each data partition using jModelTest 2.1.5 under the Akaike Information Criterion.

Genetic marker	Selected evolutionary model		
	TIM + I + G		
16S	1st codon position	2nd codon position	3rd codon position
CAD	TVM + I + G	TPM3uf + G	TIM2 + G
COI	GTR + I + G	TVM + I + G	TIM2 + G
EF1a	TIM2 + I + G	TIM3 + I + G	TVM + I
PER	TrNef + I + G	TIM2 + I + G	TPM2uf + I + G
PGD	GTR + I + G	TPM3uf + i	TrN + G

lein). The rRNA 16S gene was aligned using the multiple alignment using fast Fourier transform (MAFFT) program (Katoh et al., 2005, 2009) version 7, which implements iterative refinement methods (Katoh and Standley, 2013). The E-INS-I strategy was chosen because it is optimized for a small-scale alignment and is recommended for sequences with multiple conserved domains and long gaps, such as rRNA genes (Katoh et al., 2009).

2.4. Phylogenetic analyses

We used two different methods of phylogenetic analysis, Maximum Likelihood (ML) and Bayesian inference (BI). For both analyses, the molecular data set was divided into 16 partitions: first, second and third codon positions of CAD, COI, EF1a, PER and PGD, and 16S gene. We determined the best choice of model for each partition using jModelTest 2.1.5 (Darrriba et al., 2012) under the Akaike Information Criterion (AIC), as recommended by Posada and Buckley (2004), and analyzed the data under the recommended models (see Table 3).

ML analyses were performed using the Genetic Algorithm for Rapid Likelihood Inference, Garli v2.1 MPI (Zwickl, 2006, 2013), on the Smithsonian Institution High Performance Cluster (SI/HPC), known as Hydra-3. Twenty-four independent runs (24 different runs with the command searchreps = 1) were conducted using the scorethreshforterm = 0.05 and significanttopchange = 0.001 settings and using the automated stopping criterion, terminating the search when the ln score remained constant for 50,000 consecutive generations. Bootstrap support values (BS) were estimated from 1000 replicates using the same independent models in Garli.

Phylogenetic estimation using the Markov Chain Monte Carlo algorithm as implemented in MrBayes 3.2.5 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was performed using a parallelized version of the software. Data were divided into the above 16 partitions and each partition has its own set of parameters. Priors were applied with default values. Six runs, with four chains each (one “cold” chain and three heated chains; temp = 0.5), were performed simultaneously for 30,000,000 generations which were sufficient to bring the convergence (average standard deviation) to a value <0.1 (Ronquist et al., 2005), sampling trees every 5000 generations. The program Tracer 1.5 (Drummond and Rambaut, 2007; Rambaut and Drummond, 2007) was used to check convergence and acceptable mixing. The initial 1500 trees (25%) were discarded as burn-in and Bayesian posterior probabilities (PP) were calculated using a 50% majority-rule consensus tree inferred from the data.

All trees were drawn with the aid of FigTree v.1.3.1 (Rambaut, 2009), Adobe Illustrator® and Adobe Photoshop® CS5.

3. Results

A total of 160 *Anastrepha* and six *Toxotrypana* specimens were successfully sequenced, representing 146 *Anastrepha* and four *Tox-*

otrypana species (13 *Anastrepha* species with two terminals of the same species, and *A. obliqua* and *T. littoralis* with three terminals each). The aligned data matrix had a total 4712 characters with 561 bp for the aligned 16S, 793 bp for CAD, 1430 bp for COI, 977 bp for EF1a, 451 bp for PER, and 500 bp for PGD. Sequences of all molecular markers were not possible to amplify for all studied taxa. Levels of missing data were greater in nuclear than mitochondrial regions (nuclear markers: PGD: 66%; EF1a: 50.8%; PER: 43.5%; CAD: 37.1%; mtDNA: 16S: 6.8%; COI-3': 6.3%; COI-5': 0.5%), but there was no apparent taxonomic bias in the distribution of missing data. We included every species with at least three gene regions in our analysis, as this approach has been shown to improve phylogenetic accuracy if the missing data are distributed evenly and are not concentrated in a few taxa (Wiens, 1998, 2006; Prevosti and Chemisquy, 2009; Jiang et al., 2014). The only exceptions made for this dataset were *Anastrepha fuscata* Norrbom & Korytkowski, *A. grandicula* Norrbom, *A. hyperacantha* Norrbom & Korytkowski, and *Hexachaeta amabilis* (Loew) with two gene regions each.

The likelihood score for the best maximum likelihood tree was -87576.370208 (Figs. 1–3). The 50% majority-rule consensus tree resulted from MrBayes analysis was consistent and concordant with the ML tree and only the Bayesian posterior probabilities are shown from the Bayesian inference together with the bootstrap support values in Figs. 2 and 3.

The present analysis was not designed to study the included outgroups, but the results agree with previous studies (Han and Ro, 2009) supporting a clade with Tephritinae (only members of Tephritini were included) as sister group of *Anastrepha* + *Toxotrypana* (other taxa in the clade with these taxa in Han and Ro (2009, fig. 3) not included here), although with low support (BS = 37; PP = 0.83). Members of the Dacinae (*Ceratitis* and *Bactrocera*) form a clade (BS = 73; PP = 0.90), as do members of the tribes Carpomyini (*Zonosemata* and *Rhagoletis*; BS = 90; PP = 0.83) and Trypetini (*Strauzia* and *Parastenopa*; BS = 47; PP = 0.98) of the Trypetinae, although some with low bootstrap support. The Trypetinae is resolved as paraphyletic in this analysis, with the Dacinae and Tephritinae arising among taxa currently included in this subfamily.

Surprisingly the genus *Hexachaeta* is not supported as monophyletic, although bootstrap values are low for the clades in which its species groups are included (Fig. 2). The represented species of *Hexachaeta* are included with those of *Pseudophorellia* and *Alujamyia* in a clade with moderate support (BS = 63; PP = 0.98), and not as the sister group of the other *Toxotrypanini* (*Anastrepha* + *Toxotrypana*). The species of the *socialis* group of the subgenus *Costamyia* are grouped with the species of the *eximia* group of *Hexachaeta s. str.*, and these taxa are grouped with the species of *Pseudophorellia*, whereas the clade forming the sister group of these taxa is comprised of the species of the *amabilis* group of the subgenus *Costamyia* grouped with the species of *Alujamyia*. Support values are high for *Alujamyia* and *Pseudophorellia* (each with BS = 100; PP = 1) as well as for the *socialis* species group (BS = 97; PP = 1) and *amabilis* species group (BS = 100; PP = 1), but not for the *eximia* species group (BS = 45; PP = 0.57).

Regarding the ingroup, *Anastrepha* + *Toxotrypana*, the overall evolutionary scenario depicted in our results is a well-supported group with many internal nodes with low or very low support, or with very short branches, although some groups have high support values (Fig. 1). The *Anastrepha* + *Toxotrypana* clade is well supported (BS = 100; PP = 0.83), as is *Toxotrypana* (BS = 100; PP = 1), but the latter is placed within *Anastrepha* as sister group of the three studied members of the *cryptostrepha* clade of the *robusta* species group (BS = 100; PP = 1) (Fig. 2). The *cryptostrepha* clade + *Toxotrypana* forms the sister group of the single studied species of the *tripunctata* species group (BS = 84; PP = 0.67). The sister

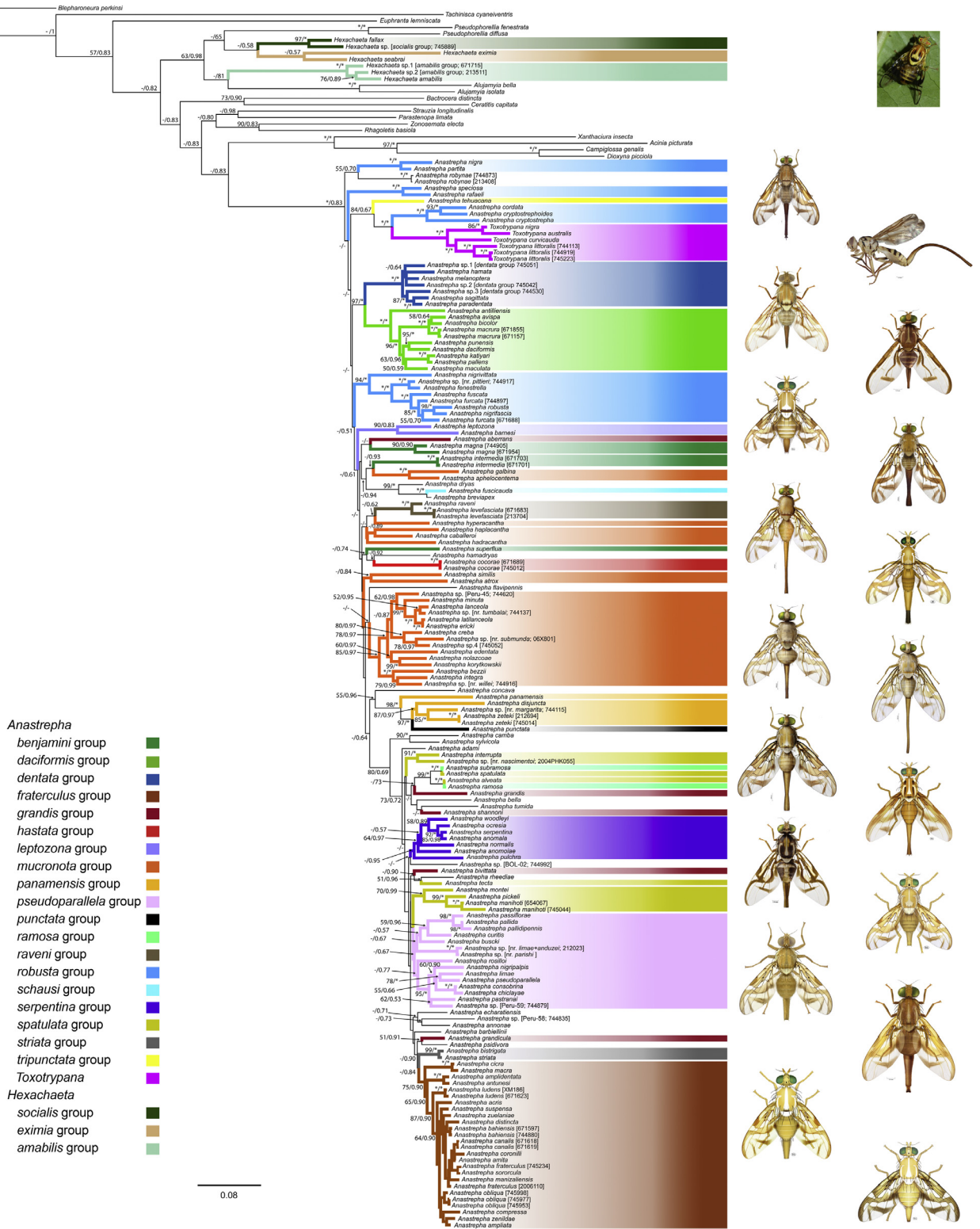


Fig. 1. Maximum Likelihood tree (Ln L = -87,576.370208) based on the combined dataset using Garli 2.1. Bootstrap support values (left) and Bayesian posterior probabilities (right) are depicted at the nodes ($\geq 50\%$ or 0.5, respectively). Asterisk (*) denotes bootstrap support values of 100% or Bayesian posterior probabilities of 1.0; a dash (-) indicates support values below 50% for bootstrap values or below 0.5 for Bayesian posterior probabilities. *Anastrepha* and *Hexachaeta* species groups are indicated using colors, even for undescribed species. Images [mostly from Norrbom et al., 2012] (right to left, top to bottom): *Hexachaeta amabilis* [credit D. Guzman], *Anastrepha robynae*, *Toxotrypana curvicauda*, *A. paradedentata*, *A. daciformis*, *A. robusta*, *A. magna*, *A. superflua*, *A. cocorae*, *A. lanceola*, *A. zeteki*, *A. ramosa*, *A. grandis*, *A. serpentina*, *A. manihoti*, *A. pseudoparallela*, *A. grandicula*, *A. fraterculus*, and *A. obliqua*.

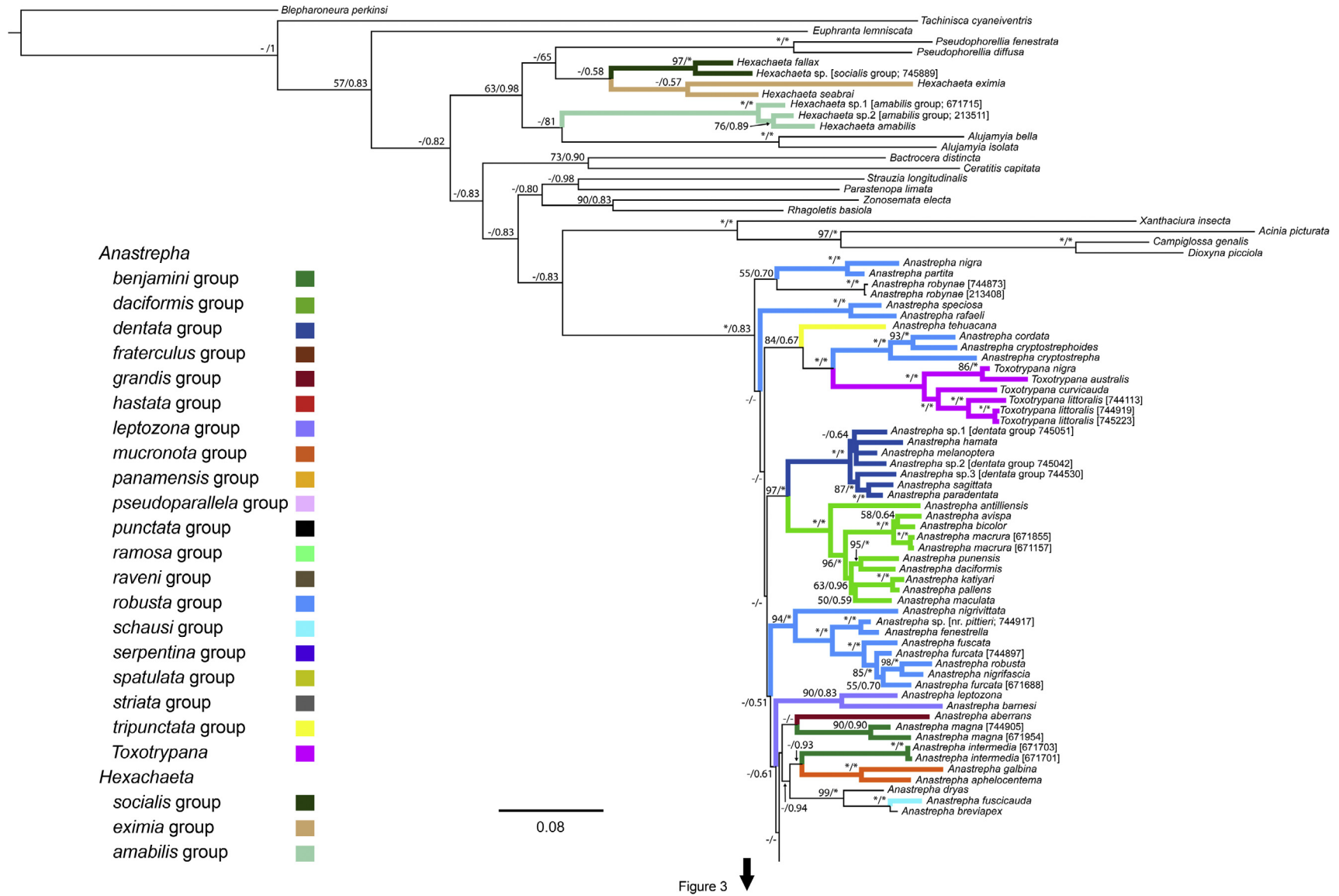


Fig. 2. Upper half (arbitrary) of the Maximum Likelihood tree (Fig. 1) based on the combined dataset using Garli 2.1. Bootstrap support values (left) and Bayesian posterior probabilities (right) are depicted at the nodes (>50%). *Anastrepha* and *Hexachaeta* species groups are indicated using colors.

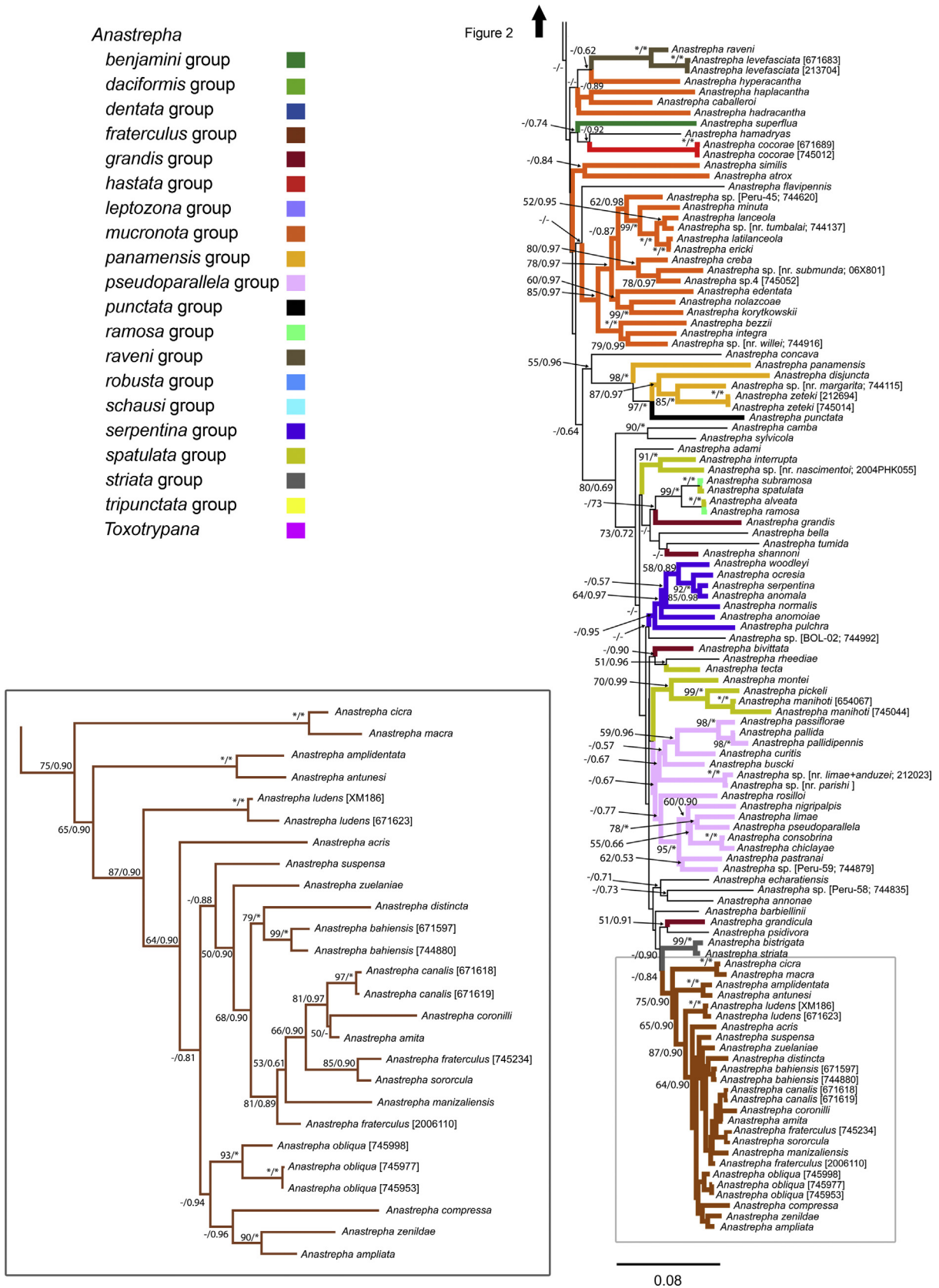


Fig. 3. Lower half (arbitrary) of the Maximum Likelihood tree (Fig. 1) based on the combined dataset using Garli 2.1. Bootstrap support values (left) and Bayesian posterior probabilities (right) are depicted at the nodes (>50%). *Anastrepha* species groups are indicated using colors. The box is an enlargement of the *fraterculus* group so that the relationships and support values are legible.

group relationship between the *daciformis* and *dentata* species groups is strongly supported (BS = 97; PP = 1.0). The large clade comprising the *pseudoparallela* + *spatulata* + *ramosa* + *doryphoros* + *grandis* + *serpentina* + *striata* + *fraterculus* groups recognized by Norrbom et al. (1999b) is recovered but without strong statistical support (BS = 73; PP = 0.72) (Fig. 3), although the inclusion of the *doryphoros* group, which is not represented in this study, could not be tested, and the inclusion of *A. bella* Norrbom & Korytkowski (previously in *robusta* group, but here considered unplaced) is unexpected. Relationships among the other species groups are not clearly resolved.

Within *Anastrepha*, of the 21 species groups recognized by Norrbom et al. (2012), two groups are not represented in this study (*caudata* and *doryphoros* groups) and four groups are represented by single species. Of the remaining 15 groups, seven are supported as monophyletic, whereas eight are recovered as non-monophyletic, although several of the latter are nearly monophyletic (except for the placement of single or a few species) (Fig. 1).

The *hastata*, *punctata*, *schausi* and *tripunctata* species groups were represented by single species in this study, thus their monophyly could not be tested. The two specimens of *A. cocorae* Norrbom & Korytkowski (*hastata* group) included in the analysis do cluster together and are placed as the sister group of *Anastrepha hamadryas* Stone, a species previously of unknown affinity (Fig. 3). The single species of the *tripunctata* group, *A. tehuacana* Norrbom, is placed as sister group of the *cryptostrepha* clade + *Toxotrypana*, as noted above (Fig. 2). The single included species of the *schausi* group, *A. fuscicauda* Norrbom & Korytkowski, is placed in a strongly-supported clade (BS = 99; PP = 1) with *A. dryas* Stone (*pseudoparallela* group) and *A. brevipapex* Norrbom, a species tentatively placed in the *mucronota* group (Norrbom et al., 2015) (Fig. 2). Another species of the *schausi* group, *A. lutea* Stone (= *A. bellicauda* Norrbom), which was not included in this study, was grouped with *A. dryas* in the 16S study by McPheron et al. (1999).

The single included species of the *punctata* group, *A. punctata* Hendel, is placed within a clade with high support values (BS = 98; PP = 1) otherwise comprised by the species of the *panamensis* group (Fig. 3).

The *daciformis* and *dentata* species groups are each strongly supported as monophyletic (both with BS = 100; PP = 1) (Fig. 2). Regarding the relationships within the *daciformis* group, the results of this study agree in some aspects with those of the morphological analysis by Norrbom (1998), e.g., the monophyly of the *macrura* complex represented by *A. avispa* Norrbom, *A. bicolor* (Stone), and *A. macrura* Hendel. They do not support the monophyly of the *daciformis* complex, however, as *A. katiyari* Norrbom is grouped with *A. pallens* Coquillett rather than *A. daciformis* Hendel. The placement of *A. antillensis* Norrbom as sister group of the other species of the group agrees with one of the hypotheses of Norrbom (1998) regarding its relationship (see his Fig. 3).

Other species groups inferred as monophyletic with high support include: the *leptozonea* group (BS = 90; PP = 0.83); the *raveni* group (BS = 100; PP = 1); and the *striata* group (BS = 99; PP = 1) (Figs. 2 and 3). The species of the *raveni* group are grouped with four species of the *mucronota* group, including the three species of the *megacantha* clade. They may be derived members of that group. The species of the *serpentina* group included in this study also form a monophyletic clade, although with relatively low support (BS = 30; PP = 0.95) (Fig. 3). A new species from Bolivia, *Anastrepha* sp. [BOL-02], is inferred as its sister group with weak support (BS = 70; PP < 0.5). The *serpentina* and *striata* groups are not recovered together, thus their combination into one group by Norrbom (2002) is not supported. The *striata* group is placed as sister group of the *fraterculus* group, although with low support (BS = 38; PP = 0.84).

One of the largest and most economically important species groups, the *fraterculus* group, is well represented in this study by 20 species (Fig. 3) and is supported as monophyletic with the exception of *A. annonae* Norrbom, which was tentatively placed in this group (Norrbom et al., 2015). This species is placed with a group of species of unknown affinity. The other 19 species form a relatively well supported cluster (BS = 75; PP = 0.90).

The *mucronota* group, the largest species group within *Anastrepha* (Norrbom et al., 2012), is well represented in this study by 24 species. The monophyly of the *mucronota* group was not supported and its members were placed in seven lineages, although the higher clades containing those lineages have low support. The majority of the species of the group (15 species) are included in a well-supported clade (BS = 85; PP = 0.97) placed as the sister group of *A. flavipennis* Greene (Fig. 3). Four species, including the three species representing the *megacantha* clade, are placed with the *raveni* group in a clade with low support. Two species (*A. galbina* Stone + *A. aphelocentema* Stone) form a well supported clade (BS = 100; PP = 1) as the sister group of *A. intermedia* Norrbom & Korytkowski (*benjamini* group) (Fig. 2), whereas two others (*A. similis* Greene + *A. atrox* (Aldrich)) form a weakly supported clade (BS = 14; PP = 0.84). The final two species, *A. robynae* Norrbom and *A. brevipapex* Norrbom, which were tentatively included in the *mucronota* group, but apparently do not belong, are placed with other species. Within the *mucronota* group, the representatives of the *lanceola* clade (Norrbom et al. 2015), *A. ericki* Norrbom, *A. lanceola* Stone, *A. latilanceola* Norrbom, *A. nr. tumbalai*, and *A. sp.* [Peru-45], form a highly supported cluster (BS = 100; PP = 1) along with *A. minuta* Stone, a somewhat similar species that previously had not been suggested to belong to the clade (Fig. 3).

The *pseudoparallela* group was well represented in this study, with ten previously included species and several more added on the basis of this study. Except for *A. dryas* Stone, these species are placed in the same clade, although it has low support (BS = 7; PP = 0.67) (Fig. 3). The clade also includes three undescribed species that belong to the group and three species, *A. buscki* Stone, *A. nigripalpis* Hendel and *A. rosilloi* Blanchard, that were not included in the group by Norrbom et al. (2012). Examination of fresh specimens of *A. rosilloi* and *A. nigripalpis*, versus the brief original descriptions used as the basis for the Norrbom et al. (2012) classification, indicates that these species also fit within the morphological definition of the group (Norrbom, personal observation), but *A. buscki* differs considerably from the other species in the form of the aculeus tip, which is nonserrate, strongly tapered, and dorsally angled. Its placement is surprising. The species of the *pallidipennis* complex (Norrbom, 1997), namely *A. curitis* Stone, *A. pallida* Norrbom and *A. pallidipennis* Greene, form a clade with good support (BS = 59; PP = 0.96).

The *robusta* species group is not supported as monophyletic in this study, but the subclades of the group recognized by Norrbom and Korytkowski (2009) that are represented are supported. The *cryptostrepha* clade, represented by *A. cryptostrepha* Hendel, *A. cryptostrephoides* Norrbom & Korytkowski and *A. cordata* Aldrich, the *nigra* clade, represented by *A. nigra* Norrbom & Korytkowski and *A. partita* Norrbom & Korytkowski, the *speciosa* clade, represented by *A. rafaelli* Norrbom & Korytkowski and *A. speciosa* Stone, the *robusta* clade, represented by *A. fenestrella* Norrbom & Korytkowski, *A. sp. nr. pittieri*, *A. furcata* Lima, *A. fuscata* Norrbom & Korytkowski, *A. nigrifascia* Stone, and *A. robusta* Greene, are each strongly supported (BS = 100; PP = 1). The *cryptostrepha* clade forms the sister group of *Toxotrypana* (Fig. 2). The *nigra* clade is the sister group of *A. robynae*, and that clade and the *speciosa* clade are the first branches within *Anastrepha*, although with low support (Fig. 2). The *robusta* clade forms the sister group of the lone representative of the *lambda* clade, *A. nigrivittata* Norrbom & Korytkowski (BS = 94; PP = 1). The two species that Norrbom and

Korytkowski (2009) left unplaced, *A. bella* Norrbom & Korytkowski and *A. concava* Greene, are placed in separate positions on the tree and do not appear to be closely related to any of the other species (Fig. 3).

Very interesting is the placement of the two studied members of the *ramosa* group, *A. ramosa* Stone and *A. subramosa* Stone, which are placed in a highly supported clade (BS = 99; PP = 1) with two species of the *spatulata* group, however, each species of the *ramosa* group is closer to one of the species of the *spatulata* group (Fig. 3).

The monophyly of the *spatulata* group is not supported by this analysis, and the members of this species group are placed in four different clades. The species feeding on the plant genus *Manihot* Mill. (Euphorbiaceae), i.e., *A. manihoti* Lima, *A. montei* Lima, and *A. pickeli* Lima, are clustered together (BS = 70; PP = 0.99). Two of the Olacaceae-feeding species (*A. alveata* Stone and *A. spatulata* Stone) are grouped with the species of the *ramosa* group (Fig. 3). Surprisingly, *A. interrupta* Stone and *A. spatulata*, which are difficult to distinguish morphologically and have the same host plant, are not inferred to be sister species.

The *benjamini* and *grandis* species groups appear to be polyphyletic. The three species of the *benjamini* group and the four species of the *grandis* group included in this study each arise in separate lineages on the tree (Figs. 2 and 3).

Some *Anastrepha* species included in this analysis were not classified in a species group by Norrbom et al. (2012, 2015). Of these 15 species, three are placed within the *pseudoparallela* group on the tree (see discussion of that group), but the rest are dispersed and none is resolved within any other recognized species groups. Most of the groupings in which these species are placed have low support values, thus their inferred relationships should only be considered as hypotheses to be further tested. One exception is the grouping of *A. camba* Norrbom and *A. sylvicola* Stone (BS = 90; PP = 1) (Fig. 3).

4. Discussion

4.1. The tribe Toxotrypanini

Han and McPheron (1997) inferred *Hexachaeta* as the possible sister group of *Anastrepha* + *Toxotrypana*, and Norrbom et al. (1999a) subsequently included it in the tribe Toxotrypanini. Conversely, Korneyev (1994) placed *Hexachaeta* in the tribe Hexachaetini, to which Korneyev (1999) added *Callistomyia* Bezzi and *Alincocallistomyia* Hardy. Korneyev (1999) also considered that the Hexachaetini were possibly related to the Xarnutini (*Xarnuta* Walker and *Platystomopsis* Hering) based mainly on nine morphological characters. All of these morphological characters are also present in some species of *Alujamyia* Norrbom, *Molynocoelia* Giglio-Tos, or *Pseudophorellia* Lima (the three genera of the *Molynocoelia* group). But none of these characters seems to be a unique synapomorphy of these three genera, *Callistomyia*, *Alincocallistomyia*, the Adramini and/or the Toxotrypanini, nor of those taxa plus the Xarnutini, as none of these characters are present in all of the species (Norrbom, 2006).

Norrbom (2006) suggested three hypotheses regarding the relationships of the *Molynocoelia* group. The first is that the group is most closely related to the Paleotropical genera *Callistomyia* and *Alincocallistomyia*, the two genera that Korneyev (1999) grouped with *Hexachaeta* in Hexachaetini. The second hypothesis is that the *Molynocoelia* group is more closely related to the Adramini, based on the usual presence of setulae on the katapimeron, a presumably apomorphic state within the Trypetinae. The third hypothesis is a close relationship between the *Molynocoelia* group and the Toxotrypanini.

The results of this study suggest that *Hexachaeta* is more closely related to at least two genera of the *Molynocoelia* group, *Pseudophorellia* and *Alujamyia*, than to *Anastrepha* and *Toxotrypana* (Figs. 1 and 2). On the other hand, the tribe Toxotrypanini *sensu* Hancock (1986; as the subfamily Toxotrypaninae) is strongly supported as monophyletic in agreement with previous morphological (Norrbom et al., 1999b) and molecular studies (Han and McPheron, 1997; McPheron et al., 1999; Han and Ro, 2009). For this reason we recognize the tribe Toxotrypanini comprising only *Toxotrypana* and *Anastrepha*. Further studies and a more complete sampling of molecular markers are needed to infer the placement of *Hexachaeta*, *Alujamyia*, *Pseudophorellia* and the *Molynocoelia* group. Our results are not conclusive as several genera (*Molynocoelia*, *Callistomyia* and *Alincocallistomyia*) were not available for this study and we were unable to sequence some nuclear markers for some of the included species.

4.2. The monophyly of *Anastrepha*

The results of this study strongly support the monophyly of *Anastrepha* + *Toxotrypana* and of the genus *Toxotrypana*. However, *Toxotrypana* is resolved within the large radiation of *Anastrepha* species. Thus, our results agree with previous studies (Norrbom et al. 1999b; McPheron et al. 1999; Barr et al. 2005) that do not support the monophyly of *Anastrepha* as currently recognized. The results indicate that *Toxotrypana* and *Anastrepha* should be considered synonyms. *Anastrepha* includes more species of economic importance and should be the valid name so as not to destabilize nomenclature for the broad community. However since *Toxotrypana* is the older name, this nomenclatural action would need to be validated by the ICZN. For this reason, we refrain from making a change here and intend to formalize the synonymy in a separate paper, once our application (in preparation) is approved by the ICZN.

4.3. Relationships among *Anastrepha* species groups

The results of this analysis agree to a large extent with previous hypotheses and the species group classification based on morphological characters (Norrbom et al., 1999b, 2012, 2015; Norrbom and Korytkowski, 2009). In general, clades that have strong morphological support are also supported here, and those with weak support are not supported by the molecular data. Most of the early branches of the cladogram are weakly supported, thus the relationships among the species groups remain largely uncertain. Exceptions include: the placement of *Toxotrypana* as sister group of the *cryptostrepha* clade (BS = 100; PP = 1), with this taxon in turn the sister group of the *tripunctata* group (or at least *A. tehuacana*, the single representative of that group included in this study (BS = 84; PP = 0.67)); the sister group relationship between the *daciformis* and *dentata* groups (BS = 97; PP = 1); and the large clade comprising the *pseudoparallela* + *spatulata* + *ramosa* + *grandis* + *serpentina* + *striata* + *fraterculus* groups (BS = 80; PP = 0.69). The relationship of the *daciformis* and *dentata* species groups and the monophyly of each of those groups are strongly supported by morphological characters (Norrbom et al. 1999b) and are very highly supported in this study. Study of the missing sequences of the current data set, additional DNA regions and addition of more species are needed to further resolve the relationships among the species groups.

Of the 15 species groups whose monophyly could be tested in this study, seven are resolved as monophyletic, whereas eight are not, although several of the latter would be monophyletic with slight adjustments (e.g., removal of a single species).

The results strongly support the monophyly of the *daciformis*, *dentata*, *leptozonea*, *raveni*, and *striata* species groups, and with

lower support, monophyly of the *serpentina* group. The *raveni* group may comprise derived members of the *mucronota* group. The hypothesis of Norrbom (2002) that the *serpentina* and *striata* groups may be closely related is not supported.

The inferred close relationship of the species of the *punctata* and *panamensis* groups is a novel result, although the single represented species of these groups in the 16S study of McPherson et al. (1999) are sister taxa in their cladogram. These groups are morphologically similar, differing by the presence/absence of paired brown markings near the posterior margin of the scutum, thus it is not surprising that molecular data support their close relationship. The position of *A. punctata* among species of the *panamensis* groups suggests that these groups might be combined. More species of the *punctata* group and further studies are needed to resolve this relationship.

The results of this analysis also support the monophyly of each of two large species groups, the *fraterculus* and *pseudoparallela* groups, with minor modification. The present study supports the notion that *Anastrepha fraterculus* (Wiedemann) as currently conceptualized is polyphyletic within the *fraterculus* species group, consistent with previous studies (Smith-Caldas et al., 2001; Hernández-Ortiz et al., 2012 and references therein; Scally et al., 2016). Our results also confirm that *A. barbiellinii* Lima should not be included in the *fraterculus* group, in agreement with Smith-Caldas et al. (2001). It was tentatively included in the group by Norrbom et al. (1999b), but not by Norrbom et al. (2012). Also, *A. annonae* Norrbom probably should be removed from the *fraterculus* group.

For the *pseudoparallela* group, additional modifications are recommended. Five species (*A. nigripalpis* and *A. rosilloi*, and three undescribed species) which fit the morphological diagnosis of the *pseudoparallela* group, should be added. Our results also suggest that *A. buscki*, which differs morphologically, also should be included in that group, but *A. dryas* should be removed. Species of the *pseudoparallela* group breed almost exclusively in species of *Passiflora* L. Discovery of the hosts of *A. buscki* would be useful to test if it truly belongs in this group. Within the group, our results support the monophyly of the *pallidipennis* complex.

The *mucronota* group, as currently conceived, is not supported as monophyletic, although 15 of the 24 species included in this study do form a well-supported clade, and four other species are placed in a weakly supported clade along with species of the *raveni* group, which suggests that the latter may be derived members of the *mucronota* group. Five other species are placed in three places on the tree relatively close to the previous two clades in a section of the tree where the branches generally are weakly supported, thus the relationships of the *mucronota* group remain poorly resolved. Further study is needed to test if it is monophyletic, or perhaps paraphyletic (e.g., the *raveni* group and a few currently unplaced species, such as *A. flavipennis* Greene, should be added). One species, *A. robynae*, is more distantly placed and appears not to belong to the *mucronota* group. Within the *mucronota* group, our results support the monophyly of the *lanceola* clade with the addition of *A. minuta*, but the status of the *megacantha* clade is uncertain.

The *robusta* species group is not recovered as monophyletic in this study, but the subclades of the group are highly supported. Our results indicate that the *cryptostrepha* clade, the *nigra* clade, the *speciosa* clade, and presumably the *binodosa* clade (the latter not represented here) should be treated as separate species groups. The *robusta* group should be restricted to the species of the *lambda* and *robusta* clades, which are sister groups. The two unplaced species of the *robusta* group, *A. bella* and *A. concava*, should be treated as unplaced to species group.

The *ramosa* and *spatulata* groups were not supported by this analysis. The three *Manihot*-feeding species of the *spatulata* group

that were included formed a clade. The species of the *ramosa* group were placed in a well-supported clade with two species of the *spatulata* group. Further study is needed to clarify the composition and relationships of these two groups, which are based mainly on the shape of the aculeus. The relationships of a number of unplaced species with somewhat similar aculeus tips (e.g., *A. rheediae* Stone, *A. nacementoi* Zucchi) also need to be evaluated.

The *benjamini* species group appears to be polyphyletic as the three species of the group included in this study arise in three separate positions on the tree. Norrbom (1997), Norrbom et al. (1999b), and Norrbom and Korytkowski (2012) previously indicated that morphological support for the group is weak. It is based mainly on a character state (produced facial carina) that may not be homologous in all of the species (the part of the carina that is produced varies) and also occurs in several species in other species groups. Nevertheless, some species within the group may be closely related, such as the species with dense microtrichia surrounding the lobe of cell cua (Norrbom et al., 2015), but none of these species were available for this study. Norrbom and Korytkowski (2012) also hypothesized that *A. gigantea* Stone, *A. intermedia* Norrbom & Korytkowski, and *A. neogigantea* Norrbom & Korytkowski are closely related, but only *A. intermedia* was available for this study, so the monophyly of this clade still needs to be tested with molecular data.

The *grandis* species group also appears to be polyphyletic. The four species of the group included in this study arise in four separate positions on the tree. Norrbom et al. (1999b) indicated that morphological support for the group is weak.

The relationships of most of the 15 *Anastrepha* species included in this analysis that were not classified in a species group by Norrbom et al. (2012, 2015) remain uncertain as they were placed in groupings with low support values. Exceptions include: three species that belong in the *pseudoparallela* group, and *A. camba* and *A. sylvicola*, which form a well-supported clade.

This study provides new data, analysis, and context for a wide sampling of diversity within *Anastrepha* (including *Toxotrypana*), the most economically important fruit fly genus in the Western Hemisphere. The challenge of continuing this work, to place all of the more than 300 species of *Anastrepha* and *Toxotrypana* into a predictive phylogenetic framework is a formidable task, however, and may perhaps never be satisfied completely. Yet our understanding of species group boundaries and their relationships becomes more important as new species of these tephritids continue to be discovered and described. Analyses of additional taxa and data are expected to improve our understanding of the relationships within this group and provide improved assessments of the potential commercial impacts of these pests.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2017.05.011>.

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