Molecular Phylogenetics and Evolution 62 (2012) 191–205

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Is the mega-diverse genus *Ocyptamus* (Diptera, Syrphidae) monophyletic? Evidence from molecular characters including the secondary structure of 28S rRNA

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ARTICLE INFO

Article history:
Received 17 March 2011
Revised 17 August 2011
Accepted 23 September 2011
Available online 29 September 2011

Keywords:
Toxomerus
Ocyptamus
Monophyly
Syrphidae
Molecular phylogeny

ABSTRACT

Phylogenetic relationships between two New World Syrphinae taxa (Diptera, Syrphidae), i.e. the highly diverse genus *Ocyptamus* and the large genus *Toxomerus*, were analysed based on molecular characters. The monophyly of both taxa was tested and the taxonomic status of included subgenera and species groups was examined. *Toxomerus* constitutes the monogeneric tribe Toxomerini with more than 140 described species, while *Ocyptamus* (tribe Syrphini) is a very diverse genus (over 300 spp.) with multiple recognised subgenera and species groups. Sequence data from three gene regions were used: the mitochondrial protein-coding gene cytochrome c oxidase subunit I (COI) and the nuclear 28S and 18S ribosomal RNA genes. The secondary structure of two expansion segments (D2, D3) of the ribosomal 28S RNA gene is presented for the family Syrphidae and used for the first time in a multiple sequence alignment. Molecular data were analysed using parsimony, maximum likelihood and Bayesian inference. *Toxomerus* was always recovered as monophyletic within *Ocyptamus*, and relationships to other New World taxa such as *Salpingogaster* (*Eosalpingogaster*) were well-supported. Only the subgenera and species groups of *Ocyptamus* were consistently recovered as monophyletic lineages, thus the apparent non-monophyly of *Ocyptamus* demands reclassification of this clade.

1. Introduction

The family Syrphidae, commonly known as flower flies or hoverflies, is one of the largest families of Diptera with more than 6100 described species (Thompson, 2010), and it is exceeded in species by only six other families (Brown, 2009). Almost a third of the species of Syrphidae are found in the Neotropical Region, and this number may increase as syrphids remain under explored in this region (Amorim, 2009). The current classification of Syrphidae recognises three subfamilies: Microdontinae, Eristalinae and Syrphinae (*sensu* Thompson and Rotheray, 1998). Almost all adult syrphids visit flowers for pollen and nectar. Syrphid larvae on the other hand are quite variable in structure, habits and feeding modes, including saprophagy, phytophagy, mycophagy and predation (Thompson and Rotheray, 1998; Thompson et al., 2010).

The subfamily Syrphinae constitutes the largest predatory radiation within the family, and comprises approximately 1800 described species. Syrphine larvae are predacious, feeding frequently on soft-bodied Hemiptera but also on the larvae of Neuroptera, Lepidoptera, Coleoptera, other Diptera, Acari, and Thysanoptera (for a review see Rojo et al., 2003). Some Neotropical species, however, are secondarily phytophagous. At least three species of the genus *Allograpta* Osten Sacken, 1875 are leaf-miners, steam-borers and pollen-feeders (Nishida et al., 2002; Weng and Rotheray, 2008; Zuijen and Nishida, in press). In the tribe Toxomerini there are two additional phytophagous syrphine species. The larvae of *Toxomerus politus* (Say, 1823), also known as "the corn-feeding syrphid fly" (Riley and Howard, 1888), feed on pollen and sap from the saccharine cells of corn (*Zea mays* L.) (Marín, 1969) and larval stages of *Toxomerus apegiensis* (Harbach, 1974) feed on pollen of a bambusoid grass (Reemer and Rotheray, 2009).

The subfamily Syrphinae is presently divided into four tribes, Bacchini, Paragini, Syrphini and Toxomerini, based on the work of Vockeroth (1992); but the monophyly of Syrphini and Bacchini is questionable based on a previous molecular analysis (Mengual et al., 2008a).

1.1. The genus Ocyptamus

Ocyptamus Macquart, 1834 is a large Syrphini genus endemic to the New World with over 300 Neotropical species and 22 Nearctic species (Thompson 1999; Rotheray et al., 2000) and constitutes the third most diverse syrphid genus after *Cheilosia* Meigen, 1822 (478

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doi:10.1016/j.ympev.2011.09.014

spp.) and *Copestylum* Macquart, 1846 (332 spp.), both members of Eristalinae (Thompson, 2010). The species diversity within *Ocyptamus* represents almost 50% of all known Neotropical species for the Syrphinae and more than 50 new species still await description (Thompson, personal communication). Species of this genus show much greater variation in colour pattern and body shape than those of any other genus of the tribe Syrphini, and they also show more variation in the male genitalia than most syrphid genera (Vockeroth, 1969).

Ocyptamus larvae have been reported as predators of a diverse array of plant pests such as aphids, soft scales, mealybugs, plant hoppers, whiteflies, and mites (see Rojo et al., 2003 for a review). The number of species with known larval feeding mode is less than 10% of the fauna and include cases of exceptional feeding behaviour (Rotheray et al., 2000; Ureña and Hanson, 2010).

The taxonomic history of this genus is confusing. Hull (1949a) provided the first comprehensive treatment of *Ocyptamus* as a subgenus of *Baccha* Fabricius, 1805. He included a series of subgenera and closely related genera such as *Leucopodella* Hull, 1949 and *Dioprosopa* Hull, 1949. However, Hull (1949b) considered *Ocyptamus* as a synonym of *Baccha*. Wirth et al. (1965) divided *Baccha* into several subgenera, including *Ocyptamus*. Vockeroth (1969) treated *Ocyptamus* in the tribe Bacchini, but placed the genera *Orphnabaccha* Hull, 1949, *Hermesomyia* Vockeroth, 1969 and *Pseudoscaeva* Vockeroth, 1969 (currently accepted as subgenera of *Ocyptamus*) in Syrphini. Later, Thompson et al. (1976) listed all the above-mentioned taxa as synonyms of *Ocyptamus*. Thompson and Zumbado (2000) treated some previously described taxa as subgenera, and Mengual et al. (2008a) recently listed some putative *Ocyptamus* subgenera.

1.2. The tribe Toxomerini

The tribe Toxomerini comprises the single genus *Toxomerus* Macquart, 1855 with approximately 150 described species (Borges and Couri, 2009; Metz and Thompson, 2001; Thompson, 1981; Thompson and Thompson, 2006). Among Syrphinae, the tribe Toxomerini has the most restricted distribution with only 16 Nearctic species and more than 140 Neotropical species (Borges and Couri, 2009). *Toxomerus* species are typically relatively small, usually about 6 mm, but some species are larger than 9 mm (see Metz and Thompson, 2001 for a review). Data about larval feeding habits of *Toxomerus* are limited (see Rojo et al., 2003).

Toxomerus taxonomy is based almost exclusively on the characteristic markings of the abdominal tergites. Each species exhibits a unique pattern, but the pattern can become obscured or lost through the extension of either dark or pale areas as demonstrated for some species (Hull, 1943; Thompson, 1981). Fortunately, the male genitalia also display differences useful for species identification (Thompson and Thompson, 2006).

Enderlein (1938) established the tribe Toxomerini for *Toxomerus* and eight other genera (a mix of taxa originally from tribes Syrphini, Bacchini and Toxomerini) but it was Vockeroth (1969) who recognised and re-classified this tribe as monogeneric. There is little doubt about the monophyly of *Toxomerus* (Thompson, 1981; Vockeroth, 1969, 1992), which is supported by synapomorphies of head shape, triangular haired process on the surstylar apodemes in the male genitalia, and presence of abdominal patterning.

There is no subgeneric classification for *Toxomerus*, although Hull (1943) noted a scheme for potentially recognising species groups within *Toxomerus* based on abdominal pattern and its evolution. Thompson (1981) provided some taxonomic notes on morphological similarities among species, which seem informative for exploration of the presence of intrageneric clusters. More recently, Metz and Thompson (2001) used body size to phenotypically divide this genus in a revision of 11 species of *Toxomerus*. In the last revision of the genus (Borges and Couri, 2009), no infrageneric groups were established or used.

Mengual et al. (2008a) presented the first molecular phylogeny of Syrphinae based on two genes (mitochondrial COI and nuclear 28S rDNA) where DNA sequences of *Toxomerus* species were included for the first time. In their cladogram Toxomerini was resolved as a monophyletic group within *Ocyptamus* and both were resolved in a separate clade from most Holarctic Syrphini. In a separate study focussing on tracing the origin of phytophagy in the genus *Allograpta*, Mengual et al. (2008b) reported the same relationship between *Ocyptamus* and *Toxomerus*, although both genera were represented by fewer species.

The purpose of this study is to explore phylogenetic relationships among these two exceptionally speciose Syrphinae genera of the New World, *Ocyptamus* and *Toxomerus*. We have substantially increased our taxon sampling and included multiple representatives of several putative subgenera of *Ocyptamus*, as well as all available species of *Toxomerus*. To perform this study we used the sequences of a large fragment of mitochondrial cytochrome c oxidase subunit I (COI), a fragment of the nuclear 18S rRNA and the region D2–D3 of the nuclear 28S rRNA genes. DNA sequence data were analysed using parsimony under direct optimisation, maximum likelihood and Bayesian inference analyses and, for the first time for Syrphidae, we used the secondary structure of the ribosomal gene 28S to align the DNA sequences.

2. Materials and methods

2.1. Taxon sampling

Table 1 lists the species included in the analysis, the collection data and the GenBank accession numbers. Criteria for taxon sampling considered the diversity of *Ocyptamus* and *Toxomerus*, trying to cover as much taxonomic diversity as possible. We constrained the Neotropical species *Syrphus shorae* Fluke, 1950 as outgroup, based on the results of Mengual et al. (2008a). We also included several other outgroup genera based on the results of Mengual et al. (2008b), namely *Allobaccha* Curran, 1928, *Allograpta*, *Asarkina* Macquart, 1834, *Episyrphus* Matsumura and Adachi, 1917, *Exallandra* Vockeroth, 1969, *Meliscaeva* Frey, 1946, *Paragus* Latreille, 1804, *Sphaerophoria* Lepeletier and Serville, 1828 and *Salpingogaster* Schiner, 1868. Thus, a total of 21 outgroup taxa were included.

For the genus *Ocyptamus* we sampled specimens of six out of 13 putative subgenera (*Hermesomyia*, *Hybobathus*, *Pelecinobaccha*, *Pipunculosyrphus*, *Orphnabaccha* and *Ocyptamus sensu stricto*) and of four species groups (*stenogaster* group, *tristis* group, *cylindricus* group and *mentor* group) proposed by Thompson (1981, 2006). The other seven *Ocyptamus* subgenera represent less than 30 described species including two monotypic subgenera (*Atylobaccha* and *Styxia*). A total of 32 species of *Ocyptamus* and 34 species of *Toxomerus* were included.

An identification key for Neotropical genera (Thompson, 2006) was used, and F.C. Thompson verified any tentative identifications. Species new to science from Costa Rica were denoted as e.g. CR-3, CR-26, etc., and were identified by F.C. Thompson (National Museum of Natural History [NMNH], Smithsonian Institution, Washington DC). Taxa referred as sp. 1, sp. 2, etc., are species new to science identified by the authors.

2.2. Laboratory protocols

Pinned and/or ethanol-preserved specimens were utilised for DNA extraction using the NucleoSpin Tissue DNA Extraction kit (Machery-Nagel, Düren, Germany) following manufacturer's instructions; samples were resuspended in 50 μl ultra-pure water.

Table 1
Taxon sampling used in the molecular analysis, including GenBank accession numbers.

Taxon	*Lab code*	Label information	Accession no COI	Accession no 28S	Accession no 18S
Allobaccha sapphirina (Wiedemann, 1830)	MZH_S87	THAILAND, Chiang Mae, IV.2001. Leg.: D. Quicke & N. Laurenne. Det.: F.C. Thompson.	EF127349	EF127430	EU409230
Allobaccha sp.	MZH_XP177	EAST TIMOR, Maliana, road verge in town. S8°58′51″ E125°13′08″. 200 m. 11-XII-2005. Leg.: M.P. van Zuijen. Det.: M.P. van Zuijen.	EU409120	EU409175	EU409229
Allograpta (*Allograpta*) *obliqua* (Say, 1823)	MZH_XP38	USA, Utah, Garfield Co., Alvoy Wash. 7 km S Escalante. 37°42.5′N 111°37.8′W. 1990 m. 29-VI-2002. Leg.: M.E. Irwin & F. Parker. Det.: F.C. Thompson.	EF127310	EF127389	EU241833
Allograpta (*Antillus*) *ascita* (Vockeroth, 1969)	MZH_XP33	DOMINICAN REPUBLIC, Pedernales Prov., P.N. Sierra de Baoruco las Abejas. 18°09.011′N 71° 37.342′W. 1150 m. 18-VI-2005. Leg. N.E. Woodley. Det.: F.C. Thompson.	EU241713	EU241761	EU241810
Allograpta (*Costarica*) *zumbadoi* Thompson, 2000	MZH_XP203	COSTA RICA, San José province, Parque Nacional Chirripó. Llano Bonito, refugio, 2550 m. 09°27′08″N 083°32′20″W. 20-IV-2005. 12:30 pm. Leg./photos: Kenji Nishida, near Centropogonis ferrugineus plants. Det.: F.C. Thompson.	EU241714	EU241762	EU241811
Allograpta (*Fazia*) *centropogonis* Nishida, 2002	MZH_S492	COSTA RICA, P.N. Tapanti, Estación La Esperanza, 2600 m, 13-I-2005. Leg.: A. Martinez. Det.: F.C. Thompson.	EF127367	EF127447	EU241816
Allograpta (*Fazia*) *micrura* (Osten Sacken, 1877)	MZH_XP183	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. 26-I-2007. N10° 20.828′ W067° 41.309′. Leg.: X. Mengual. Det.: X. Mengual.	EU241723	EU241771	EU241821
Allograpta (*Rhinoprosopa*) *flavophylla* (Hull, 1943)	MZH_XP79	COSTA RICA, P.N. Tapantí, Site 2. 11-I-2005. 1500 m. Leg.: G. Ståhls. Det. X. Mengual.	EU241729	EU241776	EU241827
Asarkina (*Asarkina*) *ericetorum* (Fabricius, 1781)	MZH_S222	KENYA, Kakamega forest, 5-XII-1995, 0°17.13′N 34°56.32′E. Leg.: Earthwatch Team 6. Det.: F.C. Thompson.	EF127353	EF127434	EU241837
Asarkina (*Asarkina*) *fulva* Hull, 1941	MZH_XP100	MADAGASCAR, Fianarantsoa Prov. Ranomafana N.P., Talatakely region. 22-XI-2004. Leg.: X. Mengual. Det. X. Mengual.	EU241738	EU241785	EU241838
Episyrphus (*Episyrphus*) *balteatus* (De Geer, 1776)	MZH_XP153	SPAIN, Alicante. P.N. Marjal Pego-Oliva, Muntanyeta Verda. 19-V-2007. Leg.: X. Mengual. Det.: X. Mengual.	EU241740	EU241788	EU241840
Exallandra cinctifacies (Speiser, 1910)	MZH_XP148	KENYA, Aberdares Nat. Park. 31-XII/14-I-2006. Malaise trap. Det.: F.C. Thompson.	EU241742	EU241790	EU241843
Meliscaeva cinctella (Zetterstedt, 1843)	MZH_S557	CZECH REPUBLIC, Bohemia PLA Jezerske mountains, Korenov, 12-VI-2005. Leg.: L. Mazanek. Det.: L. Mazanek.	EU241743	EU241791	EU241845
Ocyptamus (*Hermesomyia*) *wulpianus* (Lynch Arribalzaga, 1891)	MZH_Y121	ARGENTINA, Jujuy prov., 36 km S Jujuy, Arroyo Las Lanzas; malaise trap in wooded, damp wash; 24°27.25′S 65°17.83′W. 1278 m., 27-X/14-XI-2003. Leg.: M.E. Irwin & F.D. Parker. Det.: F.C. Thompson.	EF127356	EF127437	EU241849
Ocyptamus (*Hybobathus*) CR-11b Thompson, in litt.	MZH_XP139	COSTA RICA, Heredia, INBioparque, 15/21-I-2005, malaise trap. Det.: F.C. Thompson.	EU409127	EU409182	EU409237
Ocyptamus (*Hybobathus*) *lineatus* (Macquart, 1846).	MZH_XP30	USA, Florida, Monroe. Big Pink Key: Long Beach. N 24°38.503′ W 081°19.953′. 04-I-2004. Leg. Stuke. Det.: F.C. Thompson.	EF127305	EF127384	EU409245
Ocyptamus (*Hybobathus*) *norina* (Curran, 1941)	MZH_XP75	COLOMBIA, Dpto Valle del Cauca. Cali, Corrg. Los Andes. Pichindé, El Faro. 1700 m. 15-II-2006. Leg.: C. Gutiérrez. Det.: X. Mengual.	EU409134	EU409189	EU409247
Ocyptamus (*Orphnabaccha*) CR-8 Thompson, in litt.	MZH_XP130	COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 2200 m. 03°29.137′N 76°33.596′W. 24-II-2006. Leg.: X. Mengual. Det.: X. Mengual.	EU409124	EU409179	EU409234
Ocyptamus (*Orphnabaccha*) CR-8b Thompson, in litt.	MZH_XP135	COLOMBIA, Dpto Valle del Cauca. Cali, km18. Cerro San Antonio. 2175 m. (radiotowers) 24-II-2006. 03°29.377′N 76°33.495′W. Leg.: X. Mengual. Det.: X. Mengual.	EU409121	EU409176	EU409231
Ocyptamus (*Orphnabaccha*) CR-9 Thompson, in litt.	MZH_XP24	COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio, 2180 m. 20-VII-2004. Leg. C. Prieto. Det.: X. Mengual.	EF127299	EF127378	EU409250
Ocyptamus (*Orphnabaccha*) trabis (Fluke, 1942)	MZH_XP83	COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 2200 m. 03°29.137′N 76°33.596′W. 24-II-2006. Leg.: X. Mengual. Det.: X. Mengual.	EU409142	EU409197	EU409258
Ocyptamus (*Pelecinobaccha*) *peruvianus* (Shannon, 1927)	MZH_Y788	PERU, Dpto Junín, Chanchamayo, La Merced. 09-VII-2008. Leg.: A. Martínez.	HQ845757	HQ845760	HQ845765
Ocyptamus (*Pipunculosyrphus*) *tiarella* (Hull, 1944)	MZH_XP176	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. 26-I-2007. N10° 20.828′ W067° 41.309′. Leg.: A. Martínez. Det.: X. Mengual.	EU241744	EU241792	EU241846
Ocyptamus aff. dimidiatus	MZH_XP114	SURINAME, Commewijne, 5 km SE Meerzorg. 05°47′12″N 55°07′59″W. 13-I-2006. Leg.: M. van Zuijen. Det.: M- van Zuijen.	EU409130	EU409185	EU409240
Ocyptamus aff. nodosus	MZH_XP134	COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 15-II-2006. 2175 m. Leg.: X. Mengual. Det.: X. Mengual.	EU409135	EU409190	EU409249
Ocyptamus aff. pandora	MZH_XP162	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. N10° 20.828′ W067° 41.309′. 26–01-2007. Leg.: X. Mengual. Det.: X. Mengual.	EU409126	EU409181	EU409236
Ocyptamus aff. stenogaster	MZH_XP170	VENEZUELA, Edo. Miranda. San Antonio de los Altos, IVIC. 1650 m. 22-I/01-II-2007 (Malaise trap). N10° 24.069′ W066° 58.667′. Leg.: X. Mengual. Det.: X. Mengual.	EU409140	EU409195	EU409256
Ocyptamus antiphates (Walker, 1849)	MZH_XP29	USA, FL: MONROE Co. Everglades NP: Mtazek Pond. 25°08.4′N 080°55.5′W. 1-I-2004. Leg.: W. van Steenis. Det.: W. van Steenis.	EF127304	EF127383	EU241847

(*continued on next page*)

Table 1 (*continued*)

Taxon	*Lab code*	Label information	Accession no COI	Accession no 28S	Accession no 18S
Ocyptamus caldus (Walker, 1852)	MZH_XP93	COLOMBIA, Dpto Caldas, Manizales. Corrg. Las Palomas, Reserva Natural Río Blanco. 18-II-2006. 2200–2500 m. 5°04′N 75°26.2′W. Leg.: X. Mengual. Det.: X. Mengual.	EU409122	EU409177	EU409232
Ocyptamus coeruleus (Williston, 1891)	MZH_XP89	COLOMBIA, Dpto. Cauca, Corrg. El Tambo, 20 De Julio. 2900 m. 6/8-III-2006. Leg.: C. Prieto. Det.: X. Mengual.	EU409138	EU409193	EU409254
Ocyptamus CR-26 Thompson, in litt.	MZH_XP133	COLOMBIA, Dpto Valle del Cauca. Cali, Corrg. Los Andes. Pichindé, El Faro. 1700 m. 15-II-2006. Leg.: X. Mengual. Det.: X. Mengual.	EU409128	EU409183	EU409238
Ocyptamus CR-29 Thompson, in litt.	MZH_XP174	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Estación Biológica Rancho Grande, 1183 m. 25-I-2007. N10° 20.994′ W067° 41.059′. Leg.: E. Arcaya. Det.: X. Mengual.	EU409125	EU409180	EU409235
Ocyptamus CR-41 Thompson, in litt.	MZH_XP86	COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 15-II-2006. 2200 m. Leg.: C. Gutiérrez. Det.: X. Mengual.	EU409133	EU409188	EU409246
Ocyptamus CR-41b Thompson, in litt.	MZH_XP138	COLOMBIA, Dpto Caldas, Manizales. Corrg. Las Palomas, Reserva Natural Río Blanco. 18-II-2006. 2200–2500 m. 5°04′N 75°26.2′W. Leg.: X. Mengual. Det.: X. Mengual.	EU409123	EU409178	EU409233
Ocyptamus dimidiatus (Fabricius, 1781)	MZH_XP191	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. 26-I-2007. N10° 20.828′ W067° 41.309′. Leg.: X. Mengual. Det.: X. Mengual.	EU409129	EU409184	EU409239
Ocyptamus fascipennis (Wiedemann, 1830)	MZH_XP222	CANADA, QC: Manicouag. Les Bergeronnes, Paradis Marin camping. N48°16′20″ W69°28′11″. Alt. 30 m. 21-VIII-2007. Leg. W. van Steenis. Det.: W. van Steenis.	EU409131	EU409186	EU409241
Ocyptamus funebris Macquart, 1834	MZH_S487	COSTA RICA, Heredia, INBioparque, 15/21-I-2005, malaise trap. Det.: F.C. Thompson.	EF127364	EF127443	EU409242
Ocyptamus fuscipennis (Say, 1823)	MZH_XP15	USA, Florida. Johns CO. Anastasia island. 29°43′N 81°15″W. 24-V-02. Leg.: M. Hauser. Det.: F.C. Thompson.	EF127294	EF501970	EU409243
Ocyptamus gastrostactus (Wiedemann, 1830)	MZH_XP105	VENEZUELA, Lara, Tarabana. 500 m. 1-VIII-2006. Leg.: E. Arcaya. Det.: X. Mengual.	EU409132	EU409187	EU409244
Ocyptamus melanorrhinus (Philippi, 1865)	MZH_Y215	CHILE, Region IV, Limari prov., Fundo Agua Amarilla, 7 km N Los Vilos; malaise in stable dunes, 28-XII-2003/8-I-2004; 58 m; 31°50.96′S, 71°29.60′W. Leg.: M.E. Irwin. Det.: F.C. Thompson.	EF127360	EF127441	EU409248
Ocyptamus sp. 1	MZH_S143	COSTA RICA, Volcan Arenal area, VIII.2001, Leg.: G. Ståhls. Det.: G. Ståhls.	EF127345	EF127427	EU409251
Ocyptamus sp. 2	MZH_XP84	COLOMBIA, Dpto Caldas, Manizales. Corrg. Las Palomas, Reserva Natural Río Blanco. 18-II-2006. 2200–2500 m. 5°04′N 75°26.2′W. Leg.: X. Mengual. Det.: X. Mengual.	EU409137	EU409192	EU409253
Ocyptamus sp. 3	MZH_XP146	COSTA RICA, INBio code1087.	EU409139	EU409194	EU409255
Ocyptamus sp. 4 (*tristis* group)	MZH_Y786	PERU, Dpto Junín, Chanchamayo, La Merced. 09-VII-2008. Leg.: S. Rojo.	HQ845758	HQ845761	HQ845766
Ocyptamus stenogaster (Williston, 1888)	MZH_XP87	COSTA RICA, INBIO Parque. 15/21-I-2005.	EU409141	EU409196	EU409257
Paragus (*Pandasyopthalmus*) *haemorrhous* Meigen, 1822	MZH_S48	SPAIN, Alicante, 2000. Leg.: S. Rojo & C. Pérez. Det.: S. Rojo.	AY174470	AY476866	EU409259
Paragus (*Paragus*) *bicolor* (Fabricius, 1794)	MZH_S108	GREECE, Lesbos island, IV-2001. Leg.: S. Rojo & C. Pérez. Det.: S. Rojo.	AY476857	AY476873	—
Salpingogaster (*Eosalpingogaster*) *conopida* (Philippi, 1865)	MZH_Y214	CHILE, Region IV, Limari prov., Fundo Agua Amarilla, 7 km N Los Vilos; malaise in stable dunes, 58 m., 31°50.96′S 71°29.60′W. 28-XII-2003/8-I-2004. Leg.: M.E. Irwin. Det.: F.C. Thompson.	EF127359	EF127440	EU241850
Salpingogaster (*Salpingogaster*) *nigra* (Schiner, 1868)	MZH_XP77	COLOMBIA, Dpto Meta, PNN Sumapaz, Cabaña Las Mirlas. 3°48′ N 73°52′ W. 29-V/19-VI-2004. 710 m. Leg.: H. Vargas. Det.: F.C. Thompson.	EU241748	EU241796	EU241853
Salpingogaster (*Salpingogaster*) *pygophora* Schiner, 1868	MZH_XP169	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. 26-I-2007. N10° 20.828′ W067° 41.309′. Leg.: G. Ståhls. Det.: X. Mengual.	EU241749	EU241797	EU241854
Sphaerophoria (*Sphaerophoria*) *loewii* Zetterstedt, 1843	MZH_S273	SWEDEN, Upplands-BrO, 15-VI-2002. Leg.: H. Bartsch. Det.: G. Ståhls.	EF127318	EF127396	EU241856
Sphaerophoria (*Sphaerophoria*) *scripta* (Linnaeus, 1758)	MZH_XP142	SPAIN, Alicante, Aspe. Partida Tolomó.07-II-2006. Leg.: P. Hurtado. Det.: X. Mengual.	EU241752	EU241800	EU241860
Toxomerus 75-5 Thompson, in litt.	MZH_XP90	COSTA RICA, Turbera "3 de Junio". 14-I-2005. 2625 m. Leg.: X. Mengual. Det.: F.C. Thompson.	EU409143	EU409198	EU409260
Toxomerus aeolus (Hull, 1942)	MZH_XP20	COSTA RICA, P.N. Tapantí. 1600 m. 12-I-2005. Leg.: F.C. Thompson. Det.: F.C. Thompson.	EU409145	EF127376	EU409262
Toxomerus aff. mutuus	MZH_XP206	COSTA RICA, San José province, Cerro de la Muerte, La Cañón near Génesis II Cloudforest Reserve. 2385 m. 09°42′23″N 083°54′35.9″W. 24-IV-2007. Leg.: K. Nishida. Det.: X. Mengual.	EU409163	EU409217	EU409280
Toxomerus anthrax (Schiner, 1868)	MZH_XP194	VENEZUELA, Edo. Miranda. San Antonio de los Altos, IVIC, Centro de Ecología. 1680–1690 m. 22-I-2007. N10° 24.069′ W066° 58.667′. Leg.: E. Arcaya. Det.: X. Mengual	EU409166	EU409220	EU409283
Toxomerus anthrax (Schiner, 1868)	MZH_XP94	COLOMBIA, Dpto Valle del Cauca. Palmira, Corrg. La Buitrera. Nirvana. 14-II-2006. 1440–1530 m. Leg.: X. Mengual. Det.: X. Mengual.	EU241753	EU241801	EU241861
Toxomerus apegiensis (Harbach, 1974)	MZH_XP184	SURINAME, Distr. Brokopondo, Brownsberg National Park. Mazaroni Trail. 04°56′45″N 55°10′59″W. 04-III-2006. Leg.: M. Reemer. Det.: M. Reemer.	EU409144	EU409199	EU409261
Toxomerus bistrigus (Bigot, 1884)	MZH_XP106	MEXICO, Colima, Villa de Álvarez, Crta Minatitlán, Colonia Burócratas.23-VIII-2006. Leg.: X. Mengual. Det.: X. Mengual.	EU409146	EU409200	EU409263
Toxomerus ciliatus (Giglio-Tos, 1892)	MZH_XP179	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Estación Biológica Rancho Grande, 1183 m. 25-I-2007. N10° 20.994′ W067° 41.059′. Leg.: X. Mengual. Det.: X. Mengual.	EU409149	EU409203	EU409266

Toxomerus costalis (Wiedemann, 1830)	MZH_XP189	SURINAME, Nassau mts. 04°48′52″N 54°36′35″W. 23-IV-2006. Leg.: M. Reemer. Det.: M. Reemer.	EU409151	EU409205	EU409268
Toxomerus CR-12 Thompson, in litt.	MZH_XP182	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. 25-I-2007. N10° 20.828′ W067° 41.309′. Leg.: S. Rojo. Det.: X. Mengual.	EU409153	EU409207	EU409270
Toxomerus CR-15 Thompson, in litt.	MZH_XP80	COSTA RICA, P.N. Tapantí. 12-I-2005. 1600 m. Det.: F.C. Thompson.	EU409154	EU409208	EU409271
Toxomerus CR-17 Thompson, in litt.	MZH_XP180	VENEZUELA, Edo. Lara. P.N. Yacambú, sector "El Blanquito", along the road. 29-I-2007. Leg.: X. Mengual. Det.: X. Mengual.	EU409155	EU409209	EU409272
Toxomerus CR-3 Thompson, in litt.	MZH_XP128	COSTA RICA, P.N. Tapantí, Site 1. 1600 m. 11-I-2005. Leg.: F.C. Thompson. Det.: F.C. Thompson.	EU409156	EU409210	EU409273
Toxomerus difficilis (Curran, 1930)	MZH_XP201	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. 26-I-2007. N10° 20.828′ W067° 41.309′. Leg.: X. Mengual. Det.: X. Mengual.	EU409147	EU409201	EU409264
Toxomerus dispar (Fabricius, 1794)	MZH_XP109	MEXICO, Colima, Villa de Álvarez, Crta Minatitlán, Colonia Burócratas.23-VIII-2006. Leg.: X. Mengual. Det.: X. Mengual.	EU409171	EU409225	EU409288
Toxomerus flaviplurus (Hall, 1927)	MZH_XP196	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Estación Biológica Rancho Grande, 1183 m. 25-I-2007. N10° 20.994′ W067° 41.059′. Leg.: X. Mengual. Det.: X. Mengual.	EU409148	EU409202	EU409265
Toxomerus floralis (Fabricius, 1798)	MZH_XP115	SURINAME, Commewijne, 5 km SE Meerzorg. 05°47′12″N 55°07′59″W. 13-I-2006. Leg.: M. van Zuijen. Det.: M. van Zuijen.	EU409157	EU409211	EU409274
Toxomerus geminatus (Say, 1823)	MZH_XP223	CANADA, QC: Manicouag. Les Bergeronnes, Paradis Marin camping. N48°16′20″ W69°28′11″. Alt. 30 m. 21-VIII-2007. Leg. W. van Steenis. Det.: W. van Steenis.	EU409158	EU409212	EU409275
Toxomerus lacrymosus (Bigot, 1884)	MZH_XP163	VENEZUELA, Edo. Miranda. San Antonio de los Altos, IVIC, Centro de Ecología. 1680–1690 m. N10° 24.069′ W066° 58.667′. 22-I-2007. Leg.: X. Mengual. Det.: X. Mengual.	EU409159	EU409213	EU409276
Toxomerus marginatus (Say, 1823)	MZH_XP217	CANADA, QC: Manicouag. Les Bergeronnes, Paradis Marin camping. N48°16′20″ W69°28′11″. Alt. 30 m. 20-VIII-2007. Leg. W. van Steenis. Det.: W. van Steenis.	EU409160	EU409214	EU409277
Toxomerus musicus (Fabricius, 1805)	MZH_XP197	VENEZUELA, Edo. Lara, Tarabana. 510 m. 28-XII-2006. Leg.: E. Arcaya & L. Romero. Det.: X. Mengual	EU409162	EU409216	EU409279
Toxomerus mutuus (Say, 1829)	MZH_XP92	COLOMBIA, Dpto Caldas, Manizales. Corrg. Las Palomas, Reserva Natural Río Blanco. 18-II-2006. 2200–2500 m. 5°04′N 75°26.2′W. Leg.: X. Mengual. Det.: X. Mengual	EU241754	EU241802	EU241862
Toxomerus norma (Curran, 1930)	MZH_XP160	VENEZUELA, Edo. Yaracuy. San Felipe, Hacienda Guáquira. Pathway, 90 m. N10° 17.848′ W068° 39.320′. 27-I-2007. Leg.: X. Mengual. Det.: X. Mengual	EU409161	EU409215	EU409278
Toxomerus ochraceus (Hull, 1942)	MZH_XP200	VENEZUELA, Edo. Miranda. San Antonio de los Altos, IVIC, Centro de Ecología. 1680–1690 m. 22-I-2007. N10° 24.069′ W066° 58.667′. Leg.: X. Mengual. Det.: X. Mengual	EU409165	EU409219	EU409282
Toxomerus pallipes (Bigot, 1884)	MZH_XP102	COSTA RICA, P.N. Tapantí, (Site 2) 11-I-2005. 1500 m. Leg.: F.C. Thompson. Det.: F.C. Thompson.	EU409167	EU409221	EU409284
Toxomerus pictus (Macquart, 1842)	MZH_XP14	COSTA RICA, INBIO Park. 14-II-2003. Det.: F.C. Thompson.	EU409168	EU409222	EU409285
Toxomerus politus (Say, 1823)	MZH_XP82	COSTA RICA, P.N. Tapantí. 12-I-2005. 1600 m. Leg.: S. Rojo. Det.: F.C. Thompson.	EU241755	EU241803	EU241863
Toxomerus procrastinatus Metz, 2001	MZH_XP213	BRAZIL, Paraná, Piraquara. Mananciais da Serra. 09-X-2006. Leg.: M.G. Hermes. Det.: L. Marinoni.	EU409170	EU409224	EU409287
Toxomerus pulchellus (Macquart, 1846)	MZH_XP13	COSTA RICA, INBIO Park. 14-II-2003. Det.: F.C. Thompson.	EU409169	EU409223	EU409286
Toxomerus rombicus (Giglio-Tos, 1892)	MZH_XP127	COSTA RICA, P.N. Tapantí, Site 1. 1600 m. 11-I-2005. Det.: X. Mengual	EU409172	EU409226	EU409289
Toxomerus sedmani Harbarch, 1984	MZH_XP198	VENEZUELA, Edo. Lara. P.N. Yacambú, sector "El Blanquito", roadside. 29-I-2007. Leg.: X. Mengual. Det.: X. Mengual	EU409164	EU409218	EU409281
Toxomerus taenius (Curran, 1930)	MZH_XP199	VENEZUELA, Edo. Miranda. San Antonio de los Altos, IVIC, Centro de Ecología. 1680–1690 m. 22-I-2007. N10° 24.069′ W066° 58.667′. Leg.: X. Mengual. Det.: X. Mengual.	EU409152	EU409206	EU409269
Toxomerus tibicen Wiedemann, 1830	MZH_XP214	BRAZIL, Paraná, Piraquara. Mananciais da Serra. 26-X-2006. Leg.: M.N. Morales. Det.: L. Marinoni.	EU409173	EU409227	EU409290
Toxomerus virgulatus (Macquart, 1850)	MZH_S79	BRAZIL, 2000. Det.: F.C. Thompson.	EF127330	EF127411	EU409291
Toxomerus virgulatus (Macquart, 1850)	MZH_XP185	SURINAME, Commewijne, 5 km SE Meerzorg. 05°47′12″N 55°07′59″W. 13-I-2006. Leg.: M. Reemer. Det.: X. Mengual.	EU409150	EU409204	EU409267
Toxomerus watsoni (Curran, 1930)	MZH_XP188	SURINAME, Distr. Para, Colareek (nr. Zanderij). 05°27′58″N 55°13′47″W. 23-III-2006. Leg.: M. Reemer. Det.: X. Mengual.	EU409174	EU409228	EU409292
Outgroup					
Syrphus shorae Fluke, 1950	MZH_XP158	VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. N10° 20.828′ W067° 41.309′. 26-I-2007. Leg.: X. Mengual. Det.: G. Ståhls.	EU409136	EU409191	EU409252

One to three legs were typically used for DNA extraction and in some cases part of or the entire abdomen was used. Remnants of specimens were preserved and labelled as DNA voucher specimens for the purpose of morphological studies and deposited at the Zoological Museum of the Finnish Museum of Natural History [MZH] and labelled as listed in Table 1.

DNA primers and PCR amplification protocols for mitochondrial COI, and nuclear 28S and 18S rRNA genes were the same as described in Mengual et al. (2008b), see Appendix A. Amplified DNA was electrophoresed on 1.5% agarose gels and purified for sequencing with the GFX PCR Purification Kit (Amersham Biotech, Little Chalfont, UK). Sequencing of PCR reactions were performed as described in Mengual et al. (2008b). The sequences were edited for base-calling errors and assembled using Sequence Navigator™ (version 1.01, Applied Biosystems). All new sequences were submitted to GenBank (see Table 1 for accession numbers).

2.3. Phylogenetic analyses

A total of 89 terminal taxa were included in the analysis. The combined molecular data of the three genes were analysed using parsimony, maximum likelihood and Bayesian inference.

2.3.1. Sequence alignment

The protein-coding COI gene was aligned manually and it was not necessary to include gaps in this alignment. The COI data matrix contained a total of 1381 nucleotide characters. The alignment of ribosomal genes 18S and 28S required a slightly different approach. The small fragment of 18S used in this analysis (350–355 bp) was aligned using the E-INS-I strategy implemented in MAFFT (Katoh et al., 2005, 2009). In contrast, the domains 2 and 3 of the 28S gene are longer and more variable in size (577–602 bp), with a very complex structure of stems and loops in their secondary structures. For this reason, the region D2-D3 of the 28S rRNA gene was aligned using the secondary structure of this gene and the methodology explained by Kjer (1995) and Kjer et al. (2009). This is the first time that the secondary structure of 28S gene is used for sequence alignment in a molecular analysis of the family Syrphidae (Fig. 1, Appendix B). As a model, we used the secondary structure of the entire 28S gene published for *Drosophila melanogaster* Meigen, 1830 (Hancock et al., 1988). Small regions of ambiguous alignment, named Regions of Expansion and Contraction (REC) by Kjer et al. (2009), were excluded from the phylogeny inference analysis. Because of this the length variability of the region D2–D3 decreased to 539–544 bp, without gaps. The combined analysis of these three genes comprised a total of 2299 bp of DNA.

2.3.2. Parsimony analysis

Phylogenetic analysis using maximum parsimony (MP) was executed using direct optimisation (DO) (Wheeler, 1996) as implemented in the computer program POY version 4.1.2 (Varón et al., 2010). DO searches for the shortest tree utilise unaligned sequences as input (Schulmeister et al. 2002; Wheeler 1996); and these sequences may be of unequal length. In our analysis, however, the utilized structurally aligned 28S data matrix was predefined as "aligned" and POY used it as provided, without optimising the insertion/deletions (indels) of the sequences. We used the default values in POY for gap:transversion/transition ratio (2:1) and implemented an heuristic search based on the approach explained in Schuh et al. (2009). Parsimony analysis was performed on a cluster with 16 Intel Xeon 1.6 GHz CPUs with 16 Gb of RAM, plus 2 of 4 Gb of RAM at the MZH involving two analytical steps. The first step consisted of 100 random addition sequence (RAS) Wagner builds with TBR branch swapping and treefusing (Goloboff, 1999) [command line: build (150) swap (trees:100) select() fuse (iterations:25, replace: best, keep 20, swap (tbr)) select()]. The resulting trees were collected and used as input trees for a second run using 150 RAS using treefusing as the base with TBR branch swapping (parameter values kept identical). The new resulting trees were used in a third run using the same parameters to ensure stability of the final trees.

The full dataset included five data partitions, three for the mitochondrial COI gene, one for the nuclear D2-D3 28S rRNA gene region and one for the nuclear 18S rRNA. COI sequences were split into three fragments as defined by the primers to speed up computation times; ribosomal genes sequences were not split in fragments. A jackknife resampling analysis (Farris et al. 1996) with 500 replicates and a probability of deletion of each character of 0.36 was applied to assess nodal support. Since resampling techniques may be meaningless under dynamic homology, different strategies can be applied. Dynamic characters can be converted to a static set, but this tends to inflate support values, as it is based on the implied alignment that favours the topology. Instead, we resampled characters by dividing the data into fragments (six fragments for COI, one fragment for 18S rRNA and three fragments for 28S rRNA) as well as the command auto_sequence_partition, which evaluates each predetermined fragment.

2.3.3. Maximum likelihood analysis

For maximum likelihood analysis we divided our dataset in five partitions: 28S gene, 18S gene, first codon position of COI, second codon position of COI and third codon position of COI. We determined the best choice of model for each partition using jModelTest 0.1.1 (Posada, 2008; Posada, 2009) under the Akaike Information Criterion (AIC), as recommended by Posada and Buckley (2004). The model chosen for 28S was TPM1uf + I + G, TPM3uf + I + G for 18S, TIM3 + G for position1, TIM2 + I + G for position 2 and TIM1 + I + G for position 3 of COI gene. We analysed the data under the recommended models using Garli–Part v0.97 (Zwickl, 2006, 2010) and conducted 40 independent runs using default settings (scorethreshforterm = 0.05; significanttopochange = 0.01) and the automated stopping criterion, terminating the search when the ln score remained constant for 20,000 consecutive generations. The tree with the highest likelihood was retained and is presented here. Analytical runs were performed on a cluster made up of 35 Apple Xserve computers (280 available CPUs), where each Xserve has two quad-core 2.93 GHz Intel Xeon processors and 12 Gb of RAM at the NMNH. Bootstrap support values (BP) were estimated from 1000 replicates using the same independent models in Garli–Part v0.97.

2.3.4. Bayesian inference

Phylogenetic estimation using the Markov Chain Monte Carlo algorithm as implemented in MrBayes 3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was performed using a parallelized version of the software using MPICH2 1.2.1p1 (http://www.mcs.anl.gov/research/projects/mpich2) on the cluster at the Smithsonian Institution. Data were divided into the above five partitions and we ran our analysis specifying a separate GTR + I + G model for each partition, where each partition has its own set of parameters. Eight runs, with four chains each (one "cold" chain and three heated chains; default values), were performed simultaneously for 40,000,000 generations which were sufficient to bring the convergence (average standard deviation) to a value < 0.006 (Ronquist et al., 2005), sampling trees every 1000 generations. The initial 10,000 trees (25%) were discarded as burn-in and clade support was calculated using Bayesian posterior probabilities (PP).

3. Results

3.1. Sequence characteristics

The mitochondrial COI dataset comprised 1381 nucleotide characters; 21 taxa were lacking a fragment of 253 nucleotides of the 5′

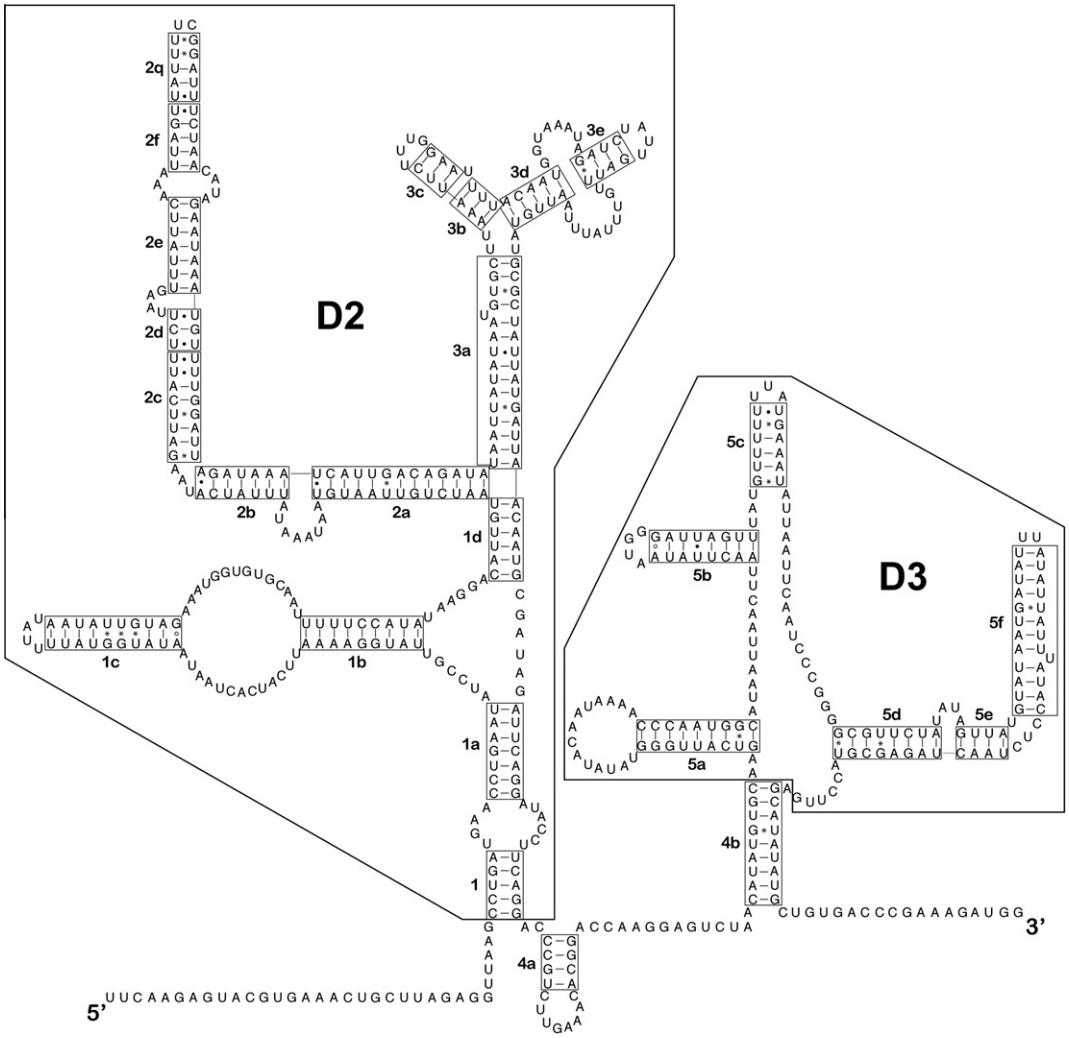

Fig. 1. The secondary structure model of the expansion segments D2 and D3 and related core sequence of the LSU 28S nuclear rRNA from the syrphid *Syrphus shorae*. Unambiguously aligned regions are boxed and notated following Gillespie et al. (2005). Base-pairing is indicated as follows: standard canonical pairs by lines (C–G, G–C, A–U, U–A); wobble G·U pairs by asterisks (G∗U); A·G pairs by open circles (A∘G); other non-canonical pairs by filled circles (e.g., U•U). The diagram was generated using the program Adobe® Illustrator.

region. The mean AT-content of the COI sequences was 72.04%. Among *Toxomerus* species the uncorrected pairwise sequence divergences for the COI gene varied between 0.0% (minimum value between ingroup taxa) between *Toxomerus aeolus* and *T. politus*, to 8.42%, between *T. ochraceus* and *T. CR-3*. In the genus *Ocyptamus*, the divergences ranged from 0.53% between *Ocyptamus* sp. 1 and *Ocyptamus dimidiatus*, to 13.59% between *Ocyptamus (Hermesomyia) wulpianus* and *O. funebris*. The sequencing of 18S rRNA produced a sequence fragment of 687 nucleotides, from which we removed a 330 nt fragment, identical among all included taxa, resulting in a 350–355 nt fragment used for analyses. This gene region could not be amplified for *Paragus* (*Paragus*) *bicolor*. The D2-D3 region of the 28S rRNA gene was amplified for all the studied terminals (see Table 1).

3.2. Parsimony analysis

The combined analysis of the three gene regions resulted in a single most parsimonious tree of length 5021 steps (CI = 0.07, RI = 0.81) shown in Fig. 2. Results identified the monophyly of the tribe Toxomerini with 1.0 jackknifing support (JS). Although there were not infrageneric groupings within *Toxomerus*, as mentioned before, several species groups were found with high support values. In contrast, the monophyly of the genus *Ocyptamus* was not supported, and four major clades were distinguished.

Ocyptamus (*Hermesomyia*) *wulpianus* was resolved alone as sister group of the clade formed by the rest of *Ocyptamus* species, *Salpingogaster (Eosalpingogaster) conopida* and *Toxomerus* species. A second clade with *Ocyptamus* species placed together the species of subgenus *Orphnabaccha* with *Ocyptamus* (*Pipunculosyrphus*) *tiarella*. The next major clade within *Ocyptamus* grouped all *cylindricus* group species.

The fourth main group of *Ocyptamus* species was the most diverse and was placed as sister group of the genus *Toxomerus*. This clade comprised *Ocyptamus CR-29* as sister group of *Salpingogaster* (*Eosalpingogaster*) *conopida*, and the subgenera *Hybobathus* and *Pelecinobaccha* with the species of the *stenogaster*, *mentor* and *tristis* groups.

Our parsimony analysis resolved the tribe Toxomerini as six major clades with relatively high support. The first group aggregated eight species (*apegiensis*, *pictus*, *pulchellus*, *politus*, *aeolus*, *lacrymosus*, *ochraceus* and *musicus*), which we called the *politus* group merely for comparison purposes in this analysis. The second clade, *watsoni* group (JS = 0.63), comprised *mutuus*, *pallipes*, *taenius*, aff. *mutuus*, *difficilis*, *CR-12* and *watsoni*. The third monophyletic group within *Toxomerus* was formed by *sedmani*, *costalis*, *CR-15*

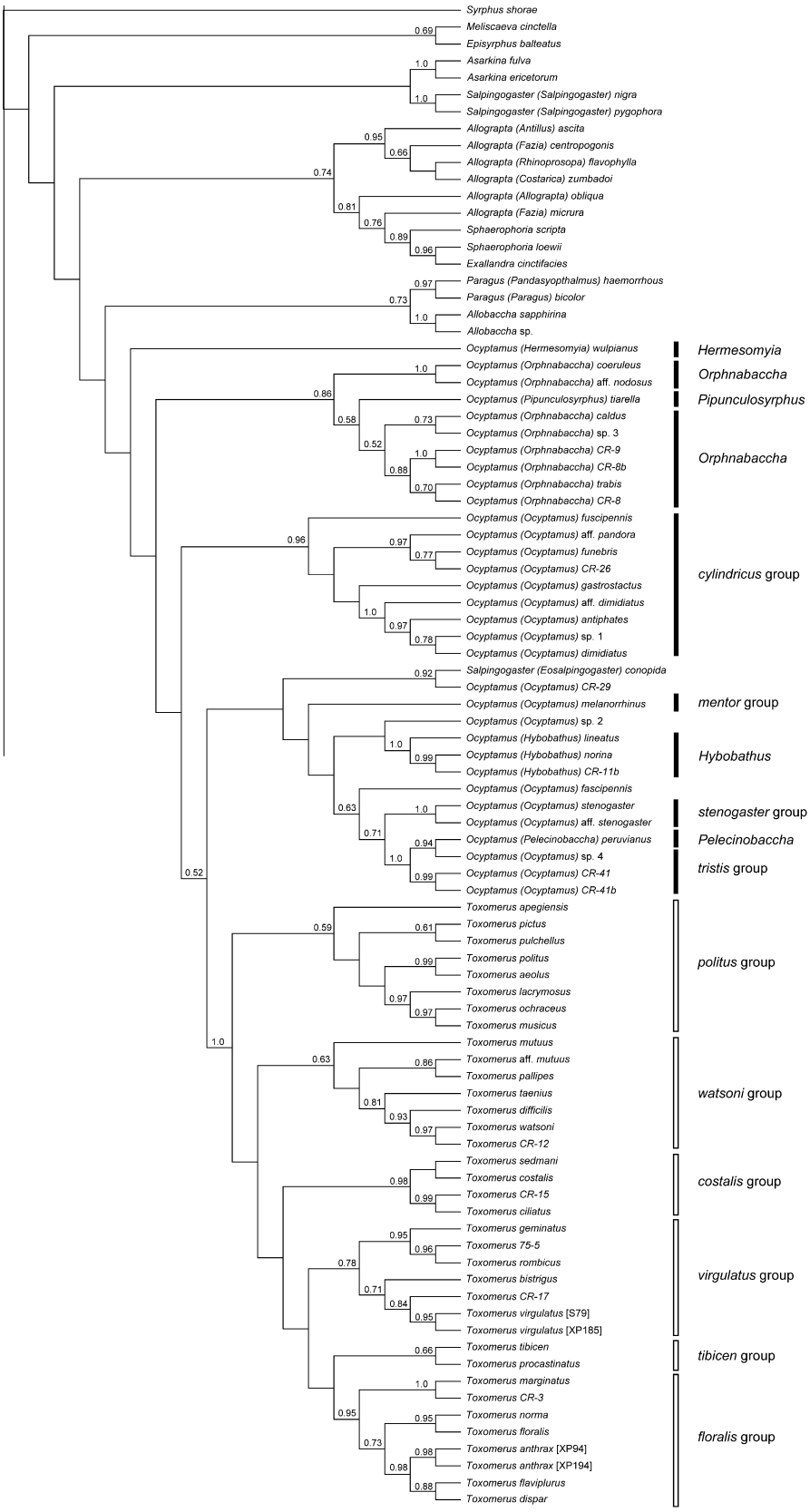

Fig. 2. Most parsimonious tree based on DNA sequences of the genes COI, 28S and 18S using POY, with the alignment of the 28S gene based on the secondary structure shown in Fig. 1. Length = 5021 steps, C.I. = 0.07, R.I. = 0.81. Gap = 2, transversion = transition = 1. Jackknife support values (>50%) are indicated above nodes. The *Toxomerus* species groups are named in order to facilitate the comparison of the results and are indicated with a grey bar. Black bars are used for recognised subgenera and species groups of *Ocyptamus*.

and *ciliatus*, named *costalis* group (JS = 0.98). The fourth clade, called *virgulatus* group (JS = 0.78), had two different subgroups with the species *geminatus*, *75–5*, *rombicus*, *bistrigus*, *CR-17* and *virgulatus*. Another smaller aggregate, *tibicen* group, (JS = 0.66) was formed by *tibicen* and *procrastinatus*. The last *Toxomerus* clade (the *floralis* group; JS = 0.95) comprised *marginatus*, *CR-3*, *norma*, *floralis*, *anthrax*, *flaviplurus* and *dispar*, although the two first species were resolved in a different clade as sister group of the rest. The other genera included in the analysis were recovered in agreement with previous results of Mengual et al. (2008a, b).

The *Toxomerus* species groups are named in order to make it easier to compare the results and to facilitate their reference in the discussion. These clades are indicated with a grey bar in Fig. 2, in contrast with the black bar used for recognising subgenera and species groups of *Ocyptamus*. We would like to add that there is still a possibility that members of one of these clades may belong to other species groups when considered in a broader phylogenetic analysis.

3.3. Maximum likelihood analysis

The likelihood score for the best ML tree (Fig. 3) was −23194.264398. The topology of the most likely tree compares favourably with the most parsimonious tree with the exception of the placement of *O. (Hermesomyia) wulpianus* and the relationships among the major groups within *Toxomerus*. The tribe Toxomerini was again recovered as monophyletic (BP = 99) within the genus *Ocyptamus*, whose monophyly was not supported.

The subgenus *Orphnabaccha* was resolved as monophyletic (BP = 90) but the relationships among the species were not identical to the ones recovered by parsimony. The *cylindricus* group was also identified as monophyletic (BP = 100) with *Ocyptamus gastrostactus* and *Ocyptamus fuscipennis* placed in different positions compared to the parsimony results. In the ML analysis *O. (Hermesomyia) wulpianus* was placed as sister group of the fourth major clade of *Ocyptamus* (including subgenera *Hybobathus* and *Pelecinobaccha*, *Ocyptamus CR-29*, *Salpingogaster* (*Eosalpingogaster*) *conopida* and the *stenogaster*, *mentor* and *tristis* groups) and the tribe Toxomerini. *Hermesomyia* was inferred to have an extremely long branch.

The species groups within *Toxomerus* defined in the parsimony results were also recovered in the ML analysis but with different relationships among them. Two major subgroups resulted: (*politus* + *watsoni*) and (*virgulatus* + (*costalis* + (*tibicen* + *floralis*))). Another minor difference with the most parsimonious cladogram is the placement of the genera *Allobaccha* and *Paragus* not as sister group of the "*Ocyptamus* clade", which includes *Ocyptamus*, *Toxomerus* and *Salpingogaster* (*Eosalpingogaster*).

3.4. Bayesian inference

The majority rule consensus tree resulting from Bayesian inference is summarised in Fig. 4. PP were high (values over 90%) in most of the branches close to the terminals, supporting species groups and subgenera. The monophyly of *Toxomerus* was again recovered with high support (PP = 1.0) and *Ocyptamus* was resolved in four main clades as in the other analyses. *Orphnabaccha* and *cylindricus* clades were recovered identically as in the ML analysis, but *O. fuscipennis* and *O. gastrostactus* are a polytomy. *Hermesomyia* was inferred with a long branch and placed as sister group of an *Ocyptamus* aggregate (*Hybobathus*, *Pelecinobaccha*, *Ocyptamus CR-29*, *Salpingogaster* (*Eosalpingogaster*) *conopida* and the *stenogaster*, *mentor* and *tristis* groups) plus *Toxomerus*, the same position that in the ML analysis. Another difference was the placement of *Ocyptamus melanorrhinus*, member of the *mentor* group, in a polytomy with other two subgroups: (*Eosalpingogaster* + *CR-29*) and (*Hybobathus* + (*tristis* + *stenogaster* + *Pelecinobaccha*)).

The major difference with MP and ML analyses is in the interrelationships of the six main clades within *Toxomerus*. Results using MrBayes showed *Toxomerus tibicen* in a basal polytomy and *T. procrastinatus* as sister group of the rest of *Toxomerus* species, but with a very low PP (0.54). Although not in the same order, the other monophyletic groups were recovered with high Bayesian posterior probabilities (1.0 for *politus*, *virgulatus*, *costalis* and *floralis* groups and 0.97 for *watsoni* group).

The position of the outgroup genera agrees with ML reconstruction placing *Allobaccha* and *Paragus* not as sister group of the "*Ocyptamus* clade". This placement disagrees with previous analysis (Mengual et al., 2008a) and with the most parsimonious tree inferred in the present study.

4. Discussion

None of the tree reconstruction methodologies supports the monophyly of the genus *Ocyptamus*, but all resolve the tribe Toxomerini as monophyletic and recognise the same species groups, although their arrangement differs. Independent of the inference method used, results showed the genus *Ocyptamus* as four clades and the genus *Toxomerus* resolved into six monophyletic groups with relatively high support values.

A minor disagreement among the methodologies is the position of the outgroup genera *Allobaccha* and *Paragus*. Neither placement (MP vs. ML and Bayesian inference) can be disputed as there is no phylogenetic hypothesis for sister group of the genus *Paragus* using larval morphology or adult morphological characters. In the last revisionary works of both genera (Dirickx, 2010; Vujić et al., 2008) there is no indication of possible sister groups. *Paragus* is the single member of the tribe Paragini and was recovered as sister group of the genus *Allobaccha* (Syrphini) in a phylogenetic analysis using maximum parsimony (Mengual et al., 2008a), in agreement with our results.

4.1. Tribe Toxomerini

Although Hull (1943) and Vockeroth (1969) did not find valuable morphological characters to subdivide the genus *Toxomerus*, our results clearly identified some species groups with high support values. Some of these groups were previously suggested, e.g. ((*aeolus* + *politus*) + *pulchellus*), the morphological similarity between *dispar* and *floralis* (resolved in the same clade), or the affinities of *difficilis* with *watsoni* (also placed in the same clade in our results) (Thompson, 1981; Thompson and Thompson, 2006).

Hull (1943, 1949b) considered *Toxomerus* as a subgenus of *Mesogramma* that included species with males having a thickened, arcuate metafemur with an elongate basal protuberance and with arcuate metatibia with the apex flared and scoop-like. He proposed this subgenus for *geminatus* and similar species. Based on his observations it is plausible that the clade (*geminatus* + (*rombicus* + *75-5*)) corresponds to another species group, although we considered them members of our *virgulatus* group due to the limited taxon sampling.

Metz and Thompson (2001) studied the larger species of *Toxomerus* and stated that the group of *T. aquilinus* Sack, 1941, *T. insignis* (Schiner, 1868), *T. intermedius* (Hull, 1949), *T. procrastinatus*, *T. saphiridiceps* (Bigot, 1884), *T. teliger* (Fluke, 1953), *T. tibicen* and *T. undecimpunctatus* (Enderlein, 1938) might represent a distinct clade within the genus. In this study *T. tibicen* and *T. procrastinatus* were resolved together as sister group of the *floralis* group, except in the Bayesian analysis where they were not resolved together. Enderlein (1938) created two names for this clade, *Mitrosphen*

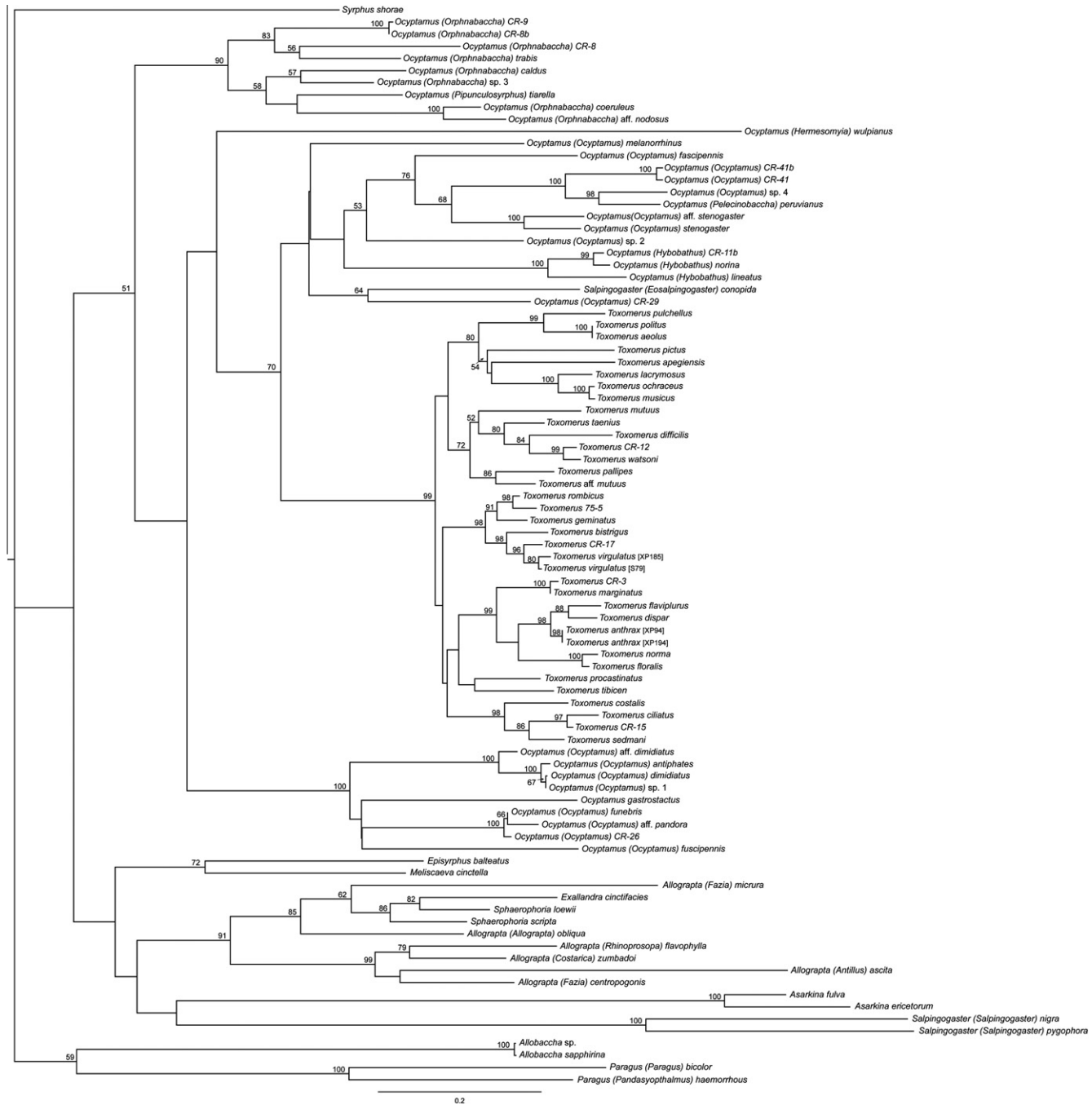

Fig. 3. Maximum Likelihood tree (ln $L = -23194.26$) based on the combined dataset using Garli–Part v0.97 and different models for each partition (TPM1uf + I + G for 28S, TPM3uf + I + G for 18S, TIM3 + G for position1, TIM2 + I + G for position 2 and TIM1 + I + G for position 3 of COI gene). Values of bootstrap support from 1000 pseudo-replicates are depicted above the nodes.

and *Antiops*, now considered synonyms of *Toxomerus*. Metz and Thompson (2001) also listed some useful morphological characters to define this group: elongate narrow frontal triangle of the male, anterior position of ocellar triangle, raised ventral area of frons in female and more sinuate R_{4+5} vein. The relative large body size of *norma* (=*mulio*), *politus* and *valdesi* (Fluke, 1950) gives them a superficially similarity to the aquilinus group, but it does not indicate relationship as *norma* and *politus* do not belong to the clade of *tibicen* + *procrastinatus* based on our results.

Toxomerus species are probably the most abundant flower flies in the New World, but relatively little biological information is recorded. Most *Toxomerus* species for which this information is available (nine species out of more than 150) are associated with common pest species, with immature stages feeding mainly on Hemiptera (families Aphididae, Delphacidae, Aleyrodidae and Pseudococcidae), Acari, Thysanoptera, Lepidoptera larvae and gall midge larvae (Cecidomyiidae) (see review in Rojo et al., 2003). The larvae of the most common and widespread Nearctic species, *Toxomerus marginatus*, are predacious on aphids and other arthropods, but Fluke (1929) and Hamrum (1966) believed that they could also feed on pollen and other plant parts. There is no published evidence to support their statements. Larvae of *T. apegiensis* was shown to feed on pollen of *Olyra obliquifolia* Steudel, a bambusoid grass (Poaceae) that grows in the understorey of

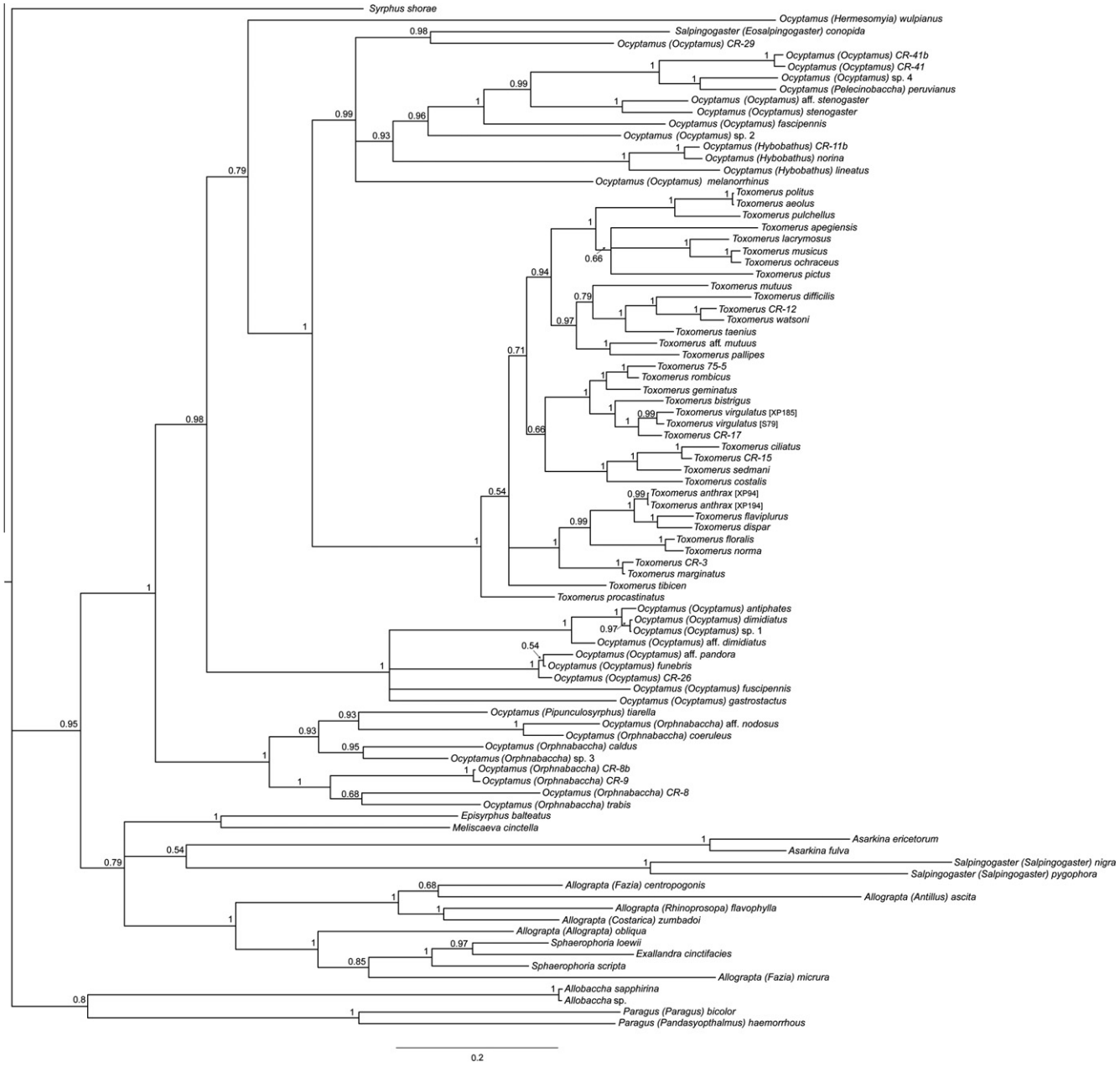

Fig. 4. Phylogenetic relationships based on Bayesian analysis of COI, 28S rDNA and 18S rDNA sequences using the evolutionary model GTR + I + G performed in MrBayes. Bayesian posterior probabilities over 50% are indicated above the nodes.

tropical rainforests (Reemer and Rotheray, 2009) and *T. politus* is a phytophagous species whose larvae feed on pollen and sap from the saccharine cells of corn (*Zea mays* L.) (Marín, 1969; Smith, 1974). Additionally, there are doubtful records of aphid-feeding for this taxon as well (Fluke, 1929; Wildermuth and Walter, 1932). In this study these two species were resolved in the same clade, the *politus* group. This placement might suggest a group comprising the pollen-feeders, although not all the species of the *politus* group appear to share this feeding mode; for instance, *Toxomerus lacrymosus* was reported feeding on Aleyrodidae (de Oliveira et al., 2003). Finally, we must point out that the feeding mode of most of the species included in the analysis is still unknown.

4.2. The genus Ocyptamus

Hull (1949a) described the genus *Orphnabaccha* based on *Baccha coeruleus* Williston, 1891 for species with pilose metasternum. Vockeroth (1969) transferred *Orphnabaccha* to Syrphini and indicated its great morphological variation, with many of its species incorrectly referred to other genera. Vockeroth divided *Orphnabaccha* in three species groups based on abdomen shape and male genitalia, although the three groups share pilose anterior anepisternum, pilose metasternum, well-developed facial tubercle and bare metaespisternum. The *coreuleus* group have black and grey abdomen, black face, darkened costal area and infuscate apical wing. The *amplus* group has a typical *Syrphus*-type abdominal pattern and furcate superior lobe of the male genitalia. The *caldus* group is more variable but members share yellow face with medial black vitta and abdominal yellow markings. Our molecular analyses recovered these three groups within *Orphnabaccha* with high support values: *caldus* and sp. 3 (*caldus* group; JS = 0.73, PP = 0.95); *trabis*, *CR-8*, *CR-9* and *CR-8b* (*amplus* group; JS = 0.88, PP = 1, BP = 83); and *coeruleus* and aff. *nodosus* (*coeruleus* group; JS = 1.0, PP = 1, BP = 100).

The subgenus *Pipunculosyrphus* was always placed within *Orphnabaccha* as a sister group to the *coeruleus* group (ML and Bayesian inference) or sister group of the *amplus* and *caldus* groups (MP). Hull (1937) described *Pipunculosyrphus* based on a new species, *globiceps*, with large head wider than the thorax, very narrow face, bare metasternum, small short abdomen with parallel sides, wing longer than the reduced abdomen and alula absent. Later, he described *tiarella* based on a female and moved *Pipunculosyrphus* under *Baccha* as a subgenus (Hull, 1944). *Ocyptamus tiarella* has pilose metasternum and a narrow alula contradicting the definition of the subgenus *Pipunculosyrphus* but agreeing with *Orphnabaccha*. Thus, *O. tiarella* must be considered member of the subgenus *Orphnabaccha*. Our analyses recovered this relationship and revealed *Orphnabaccha* as a monophyletic group considering this new addition (JS = 0.86, PP = 1, BP = 90).

The *cylindricus* group as defined here comprises the majority of the species included in the *funebris* group *sensu* Hull (1949a). Representatives of this group have broad microtrichose alula, bare metasternum, pilose metaepisternum ventrad to spiracle, uniformly dark metatarsus, parallel-sided abdomen mostly black and wing extensively dark, infuscate. The principal distinction of this group is a well-developed collar of pili along the anterior margin of the scutum, but this character is shared by other species in other groups (Hull, 1949a; Shannon and Aubertin, 1933). Our results supported the monophyly of this group (JS = 0.96, BP = 100, PP = 1). The known larvae of the species within this group (*antiphates*, *cylindricus*, *dimidiatus*, *fasciatus*, *funebris*, *fuscipennis* and *gastrostactus*) feed only on aphids (Aphididae) (see Rojo et al., 2003). Hull (1949a) considered *fascipennis* as member of the *cylindricus* group but our molecular analyses did not support this relationship, nor does its known natural history (*fascipennis* larvae feed on mealybugs, Pseudococcidae) (Rojo et al., 2003). Hull (1949a) also included in this group *Ocyptamus lemur* (Osten Sacken, 1877), whose larvae have also been reported feeding on Pseudococcidae. *Ocyptamus fascipennis* and *O. lemur* are Nearctic species (the former found from Manitoba to Quebec southwards to Texas and Florida, and the latter from British Columbia to Alberta southwards to California and Texas) and both have the second costal cell hyaline and a long subpetiolate abdomen. We think *lemur* and *fascipennis* might constitute a different species group.

Ocyptamus wulpianus is a very distinctive species described several times as a new species or a new genus (summarised in Rotheray et al., 2000). Hull (1949a) recognised a small group of species within his subgenus *Aulacibaccha* characterised by the parallel-sided abdomen with transverse bands, the *pirata* group, and included the species *Baccha pirata* Curran, 1939 and *Baccha phobifer* Hull, 1943, both junior synonyms of *wulpianus* (Rotheray et al., 2000). Vockeroth (1969) designated a new genus, *Hermesomyia*, for a new species called *bacchiformis* (junior synonym of *wulpianus*) and pointed out that *Hermesomyia* and *Pseudoscaeva* (another new genus closely related with *Hermesomyia* and currently synonym of *Ocyptamus*) were possibly derived from the same ancestor as the *Orphnabaccha* species. For Vockeroth, *Hermesomyia* is well-defined by having metaepisternum with a tuft of long fine pili ventrad of spiracle, reduced anal lobe and alula of wing and a well-developed 5th abdominal segment with tergum and sternum subquadrate (a unique character among Syrphini). Our molecular analyses reported two different placements for *wulpianus*, where the position reported by ML and Bayesian inference is similar to the one inferred by previous parsimony analysis with less taxa (Mengual et al., 2008a). Previous parsimony analyses with the present dataset placed *wulpianus* in different positions in suboptimal cladograms confirming that its placement is unstable. Larvae of *Hermesomyia* live in the water-filled leaves of bromeliads and have been reported feeding on soft-bodied larvae of craneflies (Tipulidae), mosquitoes (Culicidae), aquatic beetles (Coleoptera, Helodidae), and larvae of other syrphids [*Ornidia obesa* (Fabricius, 1775) and *Quichuana angustiventris* (Macquart, 1855)] under artificial rearing conditions (Rotheray et al., 2000). Rotheray et al. (2000) suggested that *O. wulpianus* and *O. luctuosus* (Bigot, 1884) use venom to subdue their prey. *O. luctuosus* is a species found in these phytotelmata and belongs to subgenus *Aulacibaccha* but not available for the present analysis. In the same work, Rotheray et al. (2000) hypothesised a single origin for aquatic predation and also proposed that *wulpianus* possesses the plesiomorphic character states for the position of suckers, arrangement of needle-like spines and length of posterior breathing tube.

Ocyptamus melanorrhinus is the only species of the *mentor* group included in this study. The *mentor* group is poorly defined and comprises species with yellow face, without alula and very slender abdomen (2nd abdominal segment five to ten times as long as it is wide). It is understudied and probably includes some species complexes. A previous molecular analysis recovered *melanorrhinus* as sister group of *Salpingogaster (Eosalpingogaster) conopida* (Mengual et al., 2008a), a position now occupied by *Ocyptamus CR-29*. Hull (1949a) divided the subgenus *Baccha sensu stricto* in two groups: *obscuricornis* and *victoria*. The *mentor* group is just a part of the *victoria* group and the *obscuricornis* group included the *stenogaster* group and related species plus the Holarctic species *Baccha elongata* (Fabricius, 1775), not related with *Ocyptamus*. The larval feeding mode of *melanorrhinus* is unknown but other species in its group, *mentor* (Curran, 1930) and *sativus* (Curran, 1941), have larvae feeding on mealybugs (Pseudococcidae) and whiteflies (Aleyrodidae) (Rojo et al., 2003).

The studied representatives of the *stenogaster* group, *stenogaster* and aff. *stenogaster*, were always recovered together as sister group of the *tristis* group and *Pelecinobaccha*. Species of this group have very slender abdomens (2nd abdominal segment six to ten times as long as it is wide), black face or with medial dark vitta, frons usually rugose and wing without alula. Larvae of *Ocyptamus stenogaster* have been reported feeding on Pseudococcidae, as well as the other two species of this group with known larval biology, *argentinus* (Curran, 1939) and *deceptor* (Curran, 1930) (Rojo et al., 2003).

The subgenus *Hybobathus* was recovered as monophyletic in all our analyses with high support values (JS = 1.0, BP = 100, PP = 1). Enderlein (1938) described the genus *Hybobathus* for his new species *quadrilineatus*. Hull (1949b) placed it under *Mesogramma* (=*Toxomerus*) arguing that the characters used by Enderlein (enlarged protuberance of the anterior frons separated from the posterior part by a groove) were not sufficient for a genus status. Later, Thompson et al. (1976) synonymised it with *Toxomerus*, but later *quadrilineatus* was included in *Ocyptamus* (Thompson, 2010). Species of this subgenus also have a collar of long pili along anterior margin of the scutum and share some general characters: metasternum bare, metaepisternum pilose ventrad spiracle, scutum yellow laterally with a dorsal pollinose vittate pattern, wing light greenish yellow and usually yellow legs. *Ocyptamus norina* has larvae feeding on jumping plant lice (Psyllidae) and thrips (Thysanoptera), and the other species of this subgenus with known larval biology, *lividus* (Schiner, 1868) and *zenia* (Curran, 1941), are also predators of Thysanoptera. These prey have not been reported for other *Ocyptamus* species (see Rojo et al., 2003).

Hull (1949a) recognised the *tristis* group as the largest *Baccha* group in the Neotropics. Members of this group have a black face (sometimes yellowish along the lateral sides), a petiolate abdomen usually black or black with metallic blue areas with reddish or yellowish markings, metasternum bare, metaepisternum pilose ventrad to spiracle, scutellum dark and metatarsus bicoloured (apical tarsomeres black and basitarsus black on basal half and white apically). Representatives of this group (*CR-41*, *CR-41b* and sp. 4) were recovered with *Ocyptamus (Pelecinobaccha) peruvianus*

in all the analyses with high support values (JS = 1.0, BP = 100, PP = 1). Shannon (1927) described a new subgenus of *Baccha*, called *Pelecinobaccha*, for his new species *peruvianus*. The only character used to separate this species in a new subgenus was "*the extraordinary elongate and slender abdomen of the female, which is cylindrical and nearly twice the length of the wing (17.5:10.5) and consists of six visible segments, all of equal length (except the first)*". The male of *peruvianus* does not show this unusual type of abdominal modification. Hull considered *Pelecinobaccha* as a different genus (1949a) or as a subgenus of *Baccha* (1949b) and placed *Ocyptamus telescopicus* (Curran, 1930) in this group (Hull, 1949a). The major difference between *peruvianus* and *telescopicus* is that the female of the former has elongated all the abdominal segments except the first and the female of *telescopicus* has elongated only the terminal segments. There are two other described species similar to *telescopicus* with also the 5th and 6th abdominal segments elongated, *eruptova* (Hull, 1943) and *stipa* (Hull, 1949). Except for the very elongate abdomen of the female, the rest of the morphological characters match perfectly the ones used to define the *tristis* group and these species of *Pelecinobaccha* are probably just a small group within the *tristis* group, with females showing this abdominal modification. Very little is known about the natural history of the *tristis* group species. *Ocyptamus costatus* (Say, 1829) is the only species of this group with known larval biology and immatures of this taxon feed on aphids (Aphididae) and scale insects (Coccidae).

As sister group to the clade comprising the *mentor*, *tristis* and *stenogaster* groups with *Hybobathus*, our analyses placed two taxa: *Ocyptamus CR-29* and *Salpingogaster (Eosalpingogaster) conopida*. Previous molecular analysis also reported *Eosalpingogaster* embedded in *Ocyptamus* as sister group of *O. melanorrhinus* (Mengual et al., 2008a). Our results show *Salpingogaster* (*Salpingogaster*) as the sister group to *Asarkina*, a position similar to the placement obtained by Mengual et al. (2008b), thus not supporting the current concept of the genus *Salpingogaster*. On the other hand, *Ocyptamus CR-29* is a new species found in Costa Rica not included in any species group. No additional information is available for this new species, or for *Ocyptamus* sp. 2, the other new species placed in this major clade of *Ocyptamus* that has been recovered in two different positions (Fig. 2 vs Figs. 3 and 4).

5. Conclusions

Regardless of the optimality criterion used, our results do not support the concept of a monophyletic mega-diverse genus *Ocyptamus*, a result that was obtained also in previous analyses albeit including fewer representatives of the genus (Mengual et al., 2008a, b). Instead, all species groups and proposed subgenera based on adult morphology were recovered as monophyletic. The close relationship between *Toxomerus* and *Ocyptamus* reported in this study and in previous molecular phylogenies (Mengual et al., 2008a, b) was also previously suggested based on morphological characters. Shatalkin (1975) stated that *Toxomerus* (as *Mesograpta*) was undoubtedly related to *Orphnabaccha*, *Hermesomyia* and *Pseudoscaeva*, which are now synonymised under *Ocyptamus*. The morphological similarity has frequently caused misidentifications and other systematic problems. Thompson (1999), in his Neotropical flower fly generic key, wrote:

> "*Ocyptamus* and *Toxomerus* are among the most specious and common taxa found in the New World. Unfortunately, the couplet distinguishing them may be difficult to use. While these genera can always be distinguished by the characters of the male genitalia, other characters may seem to overlap. Species of *Ocyptamus*, *Calostigma* species group, for example, are usually misidentified as *Toxomerus*. Even the experts have made mistakes. Hull described one *Toxomerus* species in what is now called *Ocyptamus*..., and Enderlein described three *Ocyptamus* species in what is now called *Toxomerus*".

Thompson (1999) used the male genitalia to define both genera. For *Ocyptamus*, male genitalia have no sclerotised process projecting between bases of surstyli, with at most a weak semi membranous process in this position, and a complex, segmented aedeagus. In *Toxomerus*, the male genitalia have a sclerotised, very short to long triangular process arising from the fused surstylar apodemes and projecting caudally between bases of surstyli, and the aedeagus is simple, unsegmented. In the present study, *Toxomerus* is recovered as monophyletic but embedded in a clade comprising *Ocyptamus* and *Salpingogaster* (*Eosalpingogaster)*. The inferred phylogeny and the placement of *Toxomerus* and *Salpingogaster* (*Eosalpingogaster)* suggest the division of the genus *Ocyptamus* into smaller groups defined by morphological characters and ecological traits, as each of the species group studied show different prey preferences. Even the subgenera not included in our analysis have a preference for different prey taxa. For example, the species *bonariensis* (Brèthes, 1905), *capitatus* (Loew, 1863) and *tristani* Zumbado, 2000 of the subgenus *Mimocalla* feed as immature on scale insects (Coccidae and Diaspididae) and whiteflies (Aleyrodidae) (Rojo et al., 2003; Thompson and Zumbado, 2000).

The use of the secondary structure of the ribosomal 28S RNA gene to align multiple sequences has been described as useful and very desirable for inferring phylogenies (Hickson et al., 2000; Kjer et al., 2009; Letsch et al., 2010). The structural alignment we present here is the first general scheme for Syrphidae (Fig. 1, Appendix B) and in this study recovered groups supported by morphology. We thus agree that the structural alignment of the 28S rRNA gene is informative and can be used not only to infer Syrphidae phylogenies but also other Diptera. Several phylogenetic studies on Hymenoptera, Coleoptera and non-Hexapodan arthropods have employed structural alignment for this gene and found it useful (Beati et al., 2004; Buffington et al., 2007; Friedrich and Tautz, 1997; Gillespie et al., 2005; Krzywinski et al., 2001; Marvaldi et al., 2009; Murienne et al., 2010).

Despite the recovered monophyly of the studied subgeneric groups, changes to the taxonomic status of the included *Ocyptamus* subgenera and species groups have not been addressed in this study because sampling is still limited. More taxa, especially unsampled subgenera, are needed to reach a conclusion about the interrelationships of the *Ocyptamus* groups, although a division of this genus seems necessary and recommendable.

Acknowledgments

The authors thank numerous researchers and collectors who made specimens available for molecular study; especially W. van Steenis, M. Reemer and M. P. van Zuijen for the amount of material they collected during field trips. We are grateful for logistical support and help in collecting material during field work to the followings entities and people: INBio (Costa Rica), IVIC, UCV and UCLA (Venezuela), Universidad del Valle (Colombia), SENASA and Universidad Nacional Agraria La Molina (Peru), Manuel Zumbado, Nancy Carrejo, Carlos Prieto, J. David Nelo, Evelin Arcaya, Graciano Tejada, Anabel Martínez-Sánchez, Yelitza Velasquez and Alexander Rodríguez. We thank CONAGEBIO for the permit no 091-2004-OFAU for insect collection for scientific study.

We especially thank Menno Reemer for kindly sharing unpublished information and F. Christian Thompson for continuous support during this study. We thank Mónica Muñoz-Torres and Wayne Mathis for valuable comments and corrections on a previous version of the manuscript and Gil Felipe Miranda for productive discussions about the genus *Ocyptamus*. We also thank Christy J. Geraci and Adriana Marvaldi for the help during the structural

alignment. This work was partially funded by grant AP2003-3998 of the Spanish Ministry of Education and Science (Ministerio Español de Educación y Ciencia) awarded to X. Mengual.

Appendixes A and B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.09.014.

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