ARTHROPOD SYSTEMATICS & PHYLOGENY

74 (2): 161 – 176
10.10.2016

SENCKENBERG

The genus *Ocyptamus* Macquart (Diptera: Syrphidae): A molecular phylogenetic analysis

Gil Felipe Gonçalves Miranda *,[1], Jeffrey H. Skevington[2], Stephen A. Marshall[3] & Scott Kelso[2]

[1] Laboratório de Entomologia Sistemática, Urbana e Forense, Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Avenida André Araujo 2936, Bairro Petrópolis, 69060-060 Manaus, Brazil; Gil Felipe Gonçalves Miranda * [gilfgm@gmail.com] — [2] Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture and Agri-Food Canada, K.W. Neatby Building, 960 Carling Avenue, K1A 0C6 Ottawa, Canada; Jeffrey H. Skevington [jhskevington@gmail.com]; Scott Kelso [scott.kelso@agr.gc.ca] — [3] University of Guelph Insect Collection and Insect Systematics Laboratory, School of Environmental Sciences, University of Guelph, Edmund C. Bovey Building, N1G 2W1 Guelph, Canada; Stephen A. Marshall [samarsha@uoguelph.ca] — * Corresponding author

Accepted 18.vii.2016.
Published online at www.senckenberg.de/arthropod-systematics on 21.ix.2016.

Editor in charge: Brian Wiegmann

Abstract

With nearly 300 described species, *Ocyptamus* Macquart, 1834 is the second most speciose genus of Syrphidae in the New World, and the most diverse genus of Syrphinae. *Ocyptamus* as a whole was last revised in the late 1940's, with many new species described after that. The genus is here placed under phylogenetic scrutiny using molecular characters from regions of the mitochondrial 12S, COI and CytB genes, and the nuclear AATS, CAD and 28S genes. *Ocyptamus* is shown to be paraphyletic with regard to *Eosalpingogaster* Hull, 1949 and *Toxomerus* Macquart, 1855. Several infra-generic taxa are supported as monophyletic and new arrangements are proposed. The relationships established in this paper indicate that there is a need for changes to the generic classification of the group.

Key words

Classification, Neotropical, *Ocyptamus*, phylogeny, Syrphidae, systematics.

1. Introduction

The Neotropical genus *Ocyptamus* is the second most speciose genus (273 spp.) in the New World, the first being *Copestylum* Macquart, 1846 with 299 spp. (Thompson et al. 2010). *Ocyptamus* species are restricted to the Americas and known larvae prey primarily on Sternorrhyncha (Hemiptera) but also feed on other gregarious phytophagous insects, invertebrates inside bromeliads and (rarely) even on flying insects (Rotheray et al. 2000; Rojo et al. 2003; Thompson et al. 2010; Ureña & Hanson 2010).

Austen (1893) separated the New World *Ocyptamus* (which he treated as *Baccha* Fabricius, 1805) into three groups: dark flies with a petiolate abdomen (group I), ferruginous / ochraceous flies with a petiolate abdomen (group II), and ferruginous / yellowish flies with a broad and flat abdomen (group III). Many species have been described since Austen's work, most significantly by Shannon (1927), and Curran and Hull during the 1930's and 1940's (Thompson et al. 1976). Shannon (1927) described the subgenus *Baccha* (*Pelecinobaccha*) for one species with a very long abdomen and segments 2 to 6 of equal length, and the genus *Calostigma* for one species with a straight M_1 vein and apical spot on the wing. Curran (1941) provided a key and described several new species.

Hull (1937, 1943, 1949) described new monobasic generic-level taxa based on small sets of distinct characters. For example, one species with large head and short abdomen became *Pipunculosyrphus* Hull, 1937, a species with a greatly inflated head and pilose eyes became *Styxia* Hull, 1943, a species with long antennal segments became *Therantha* Hull, 1943 and a species with a flat face became *Atylobaccha* Hull, 1949. Hull (1943) erected *Mimocalla* for those *Baccha* with sinuate veins R_{4+5} and M_1 and an unusually prominent frons, and compared it to *Salpingogaster* Schiner, 1868. Hull (1949) further divided the New World *Baccha* into several subgenera and species groups, proposed new genera closely related to *Baccha*, and added more species, with little to no explanation, to some genera such as *Calostigma*, *Pelecinobaccha* and *Pipunculosyrphus*. Hull (1949) defined his new subgenus *Aulacibaccha* on being 'distinctly emarginate upon at least the fifth and fourth abdominal segments' and further stated that 'The flies of *Aulacibaccha* contain the *obsoleta* group', which was further distinguished by the petiolate abdomen and contrasting ocellar triangle, and also briefly described the groups *cultrata* and *pirata* in the sequence but not explicitly relating them to *Aulacibaccha*.

It was not until twenty years after the large work of Hull (1949) that the New World *Baccha sensu lato* were studied in more depth. Vockeroth (1969) described two new genera with similar male genitalia, *Hermesomyia* and *Pseudoscaeva*, which he considered to be close to and possibly even congeneric with *Orphnabaccha* Hull, 1949, even though *Hermesomyia* had a Bacchini-like abdomen. Thompson et al. (1976) transferred all New World species of *Baccha sensu* Hull (1949), with the exception of *B. elongata* (Fabricius, 1775), to *Ocyptamus*. This included the subgenera *Aulacibaccha*, *Mimocalla*, *Pelecinobaccha*, *Styxia*, *Therantha* and *Leucopodella* (*Atylobaccha*). Additionally, Thompson et al. (1976) treated the genera *Callisyrphus* Frey, 1946, *Calostigma*, *Hermesomyia*, *Orphnabaccha*, *Pipunculosyrphus* and *Pseudoscaeva* as synonyms of *Ocyptamus*. Thompson's (1981) treatment of the Caribbean fauna expanded on Hull's (1949) group definitions by presenting a more extensive diagnosis for each group (as *Ocyptamus* species groups), not all of Hull's groups were covered because they do not all occur in the Caribbean. Miranda et al. (2014) removed *Pelecinobaccha* and *Atylobaccha* from *Ocyptamus*, elevated them to genus, and described the new genus *Relictanum* Miranda, 2014. To date, the only other part of *Ocyptamus* that has been fully reviewed is *O.* (*Mimocalla*) (Thompson & Zumbado 2000).

These past attempts to organize the classification within the genus, in the absence of a phylogenetic framework resulted in a large, inadequately defined genus divided into subgenera and species groups of dubious value and validity. Some recent phylogenetic studies suggest that *Ocyptamus* is paraphyletic relative to the genera *Toxomerus* Macquart, 1855 and *Eosalpingogaster* Hull, 1949 (Mengual et al. 2008, 2012; Mengual & Thompson 2011) and that the limits of the genus should therefore be re-evaluated. Mengual et al. (2012) produced a phylogenetic hypothesis using data from COI, 18S and 28S genes that incorporated taxa from across the *Ocyptamus* grade. They obtained support for the infra-generic groups they included in their study, however they had not included all previously recognized and named *Ocyptamus* subgroups (Table 1). One of the lineages recovered by Mengual et al. (2012) was the *Ocyptamus cylindricus* group, which includes the type species of *Ocyptamus*. There are currently 15 recognized supraspecific taxa, their rank varying depending on the author, in or related to *Ocyptamus* (Table 1) (Hull 1949; Mengual et al. 2008, 2012; Thompson 1981; Thompson, pers. comm.; Vockeroth 1969).

The current paper is the first of a two-part study that aims to review the current *Ocyptamus* classification by first identifying natural groups and their relationships, and then by naming and diagnosing them. Here we test the monophyly of the taxa that are/were part of *Ocyptamus* and explore their relationships, through parsimony and model based phylogenetic analyses using a more extensive molecular dataset and a broader sample of taxa than that used in previous studies, resurrect names for groups that are recovered as natural, and redefine *Ocyptamus sensu stricto*. A second paper is planned to add morphological data to the analysis, to add taxa for which molecular data are not available, to diagnose and name missing groups, and to present a key for identification of the named groups.

2. Material and methods

2.1. Specimens

Fresh specimens were collected from Costa Rica and Brazil, and preserved specimens borrowed from the Canadian National Collection of Insects, Arachnids and Nematodes (CNC, Ottawa, Canada) and the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK, Bonn, Germany). Costa Rican specimens were later deposited at the Instituto Nacional de Biodiversidad (INBio, San José, Costa Rica), and Brazilian specimens were deposited at the Coleção Entomológica Padre Jesus Santiago Moure (DZUP, Curitiba, Brazil) and Museu de Zoologia de São Paulo (MZSP, São Paulo, Brazil) (Electronic Supplement 1).

The *Ocyptamus* species groups, or related taxa, used as an initial framework for this study were based on previous literature (Hull 1949; Thompson 1981; Vockeroth 1969), and the names of these species groups, standardized for this two-part study, are given in Table 1. Outgroup taxa were chosen on the basis of recent phylogenetic studies (Mengual et al. 2008, 2012). A representative selection of 34 species of *Ocyptamus*, 12 of *Pelecinobaccha*, 2 of *Relictanum*, 2 of *Toxomerus*, one of *Atylobaccha* and one of *Eosalpingogaster*, plus 9 outgroup taxa were stud-

Table 1. *Ocyptamus* species groups and related genera as currently understood, and their treatment in past major works.

Sampling	***Ocyptamus* species group name & related genera**	**Hull 1949**	**Vockeroth 1969**	**Thompson 1981 *Ocyptamus* group**	**Mengual et al. 2012**
Taxa sampled in the current study	Group ***amplus***	*Orphnabaccha*	*Orphnabaccha*	species group *caldus*	*Orphnabaccha* (incl. species groups *amplus*, *caldus* and *coeruleus*)
	Group ***arx***	*Baccha* (*Aulacibaccha*), species group *obsoleta*	not treated	not treated	not treated
	Group ***Atylobaccha***	*Leucopodella* (*Atylobaccha)*	not treated	not treated	not treated, but considered a subgenus
	Group ***cylindricus***	*Baccha* (*Ocyptamus*), species group *funebris*	not treated	species group *cylindricus*	*Ocyptamus* s.str. and species group *cylindricus*
	Group ***eblis***	*Styxia*	not treated	not treated	not treated, but considered a subgenus
	Group ***elnora***	*Calostigma*	not treated	species group *elnora*	not treated
	Group ***globiceps***	*Baccha* (*Pipunculosyrphus*)	not treated	not treated	*Pipunculosyrphus*
	Group ***lepidus***	*Baccha*, species group *cultrata* and *lepidus*	not treated	species group *lepidus*	not treated
	Group ***lineatus***	*Baccha*, species group *lineatus*	not treated	species group *lineatus*	*Hybobathus*
	Group ***Pelecinobaccha***	*Pelecinobaccha*	not treated	not treated	*Pelecinobaccha* and species group *tristis*
	Group ***Relictanum***	*Baccha*, species group *tristis*	not treated	not treated	species group *tristis*
	Group ***stenogaster***	*Baccha*, species group *obscuricornis* and *victoria*	not treated	species group *stenogaster*	species group *stenogaster*
	Group ***wulpianus***	*Baccha*, species group *pirata*	*Hermesomyia*	not treated	*Hermesomyia*
Taxa not sampled in the current study	Group ***Ocyptamus* (*Mimocalla*)**	*Baccha* (*Mimocalla*)	not treated	not treated	not treated, but considered a subgenus
	Group ***parvicornis***	*Baccha*, species group *victoria*	not treated	species group *parvicornis*	not treated
	Group ***Pseudoscaeva*** (currently a synonym of *Ocyptamus*)	not treated	*Pseudoscaeva*	not treated	not treated, but considered as a synonym of *Ocyptamus*
	Group ***Therantha*** (currently a synonym of *Ocyptamus*)	*Therantha*	not treated	not treated	not treated

ied (Electronic Supplement 2). Table 1 summarizes the treatment of these groups in previous studies.

Specimens for the molecular study were collected by sweep netting or hand-collecting, preserved in 90–95% ethanol, and placed in a –20°C or –80°C freezer until extraction. The voucher data and unique identifiers for the specimens used in this study are presented in Electronic Supplement 1.

2.2. DNA extraction, PCR amplification and sequencing

Genomic DNA extractions were obtained with the QIAGEN DNeasy kit (Qiagen Inc., Santa Clara, CA, USA). Following extraction, specimens were critical-point dried or dehydrated through three successive 24 h baths of ethyl acetate and then air dried.

The target genes were chosen on the basis of sequencing success and phylogenetic utility in previous studies (Gibson et al. 2010a; Gibson et al. 2011; Mengual et al. 2008, 2012; Moulton & Wiegmann 2004).

The sequences used in this study were fragments of the mitochondrial genes 12S ribosomal DNA (12S, the first half from the 5' end), Cytochrome *b* (CytB, about three quarters from the 3' end) and Cytochrome *c* oxidase subunit I (COI) and the nuclear genes 28S ribosomal DNA (28S, covering the D1 to D3 regions), Alanyl-tRNA Synthetase (AATS, the first half from the 5' end) and of the carbamoyl phosphate synthase domain of CAD (CAD, from base position 560 to 3180). Oligonucleotides (primers) used in this study are listed in Table 2. The number of base pairs for each fragment is presented in Table 3. Gibson et al. (2011) summarize the history of the use of these genes and their related primers (and their positions on the genes) for phylogenetic analyses of Diptera.

Amplification, purification, sequencing and contig assembly were carried out as described in Gibson et al. (2010a,b). Base frequencies were calculated in PAUP* 4.0b10 (Swofford 2003).

It was not possible to obtain a full molecular dataset for some taxa (Electronic Supplement 2). The 28S and COI sequences for *Eosalpingogaster conopida* (Philippi, 1865), *Ocyptamus gastrostactus* (Wiedemann, 1830), *O. tiarella* (Hull, 1944) and *O. wulpianus* (Lynch-Arribálzaga, 1891), from Mengual et al. (2008), were obtained from the NCBI Entrez Taxonomy webpage. The remaining molecular data were newly acquired for this study. GenBank accession numbers for the sequences are given in the Electronic Supplement 1.

Table 2. List of oligonucleotides (primers) used in this study and their gene location, orientation, name, and nucleotide sequence.

Gene location	Gene	Orientation	Primer name	Sequence 5'-> 3'
Mitochondrial	12S	Forward	12SBi	AAGAGCGACGGGCGATGTGT
		Reverse	12SAi	AAACTAGGATTAGATACCCTATTAT
	COI	Forward	COI-Dipt-2411F	GCHACWATAATTATTGCHGTNCC
			LCO1490	GGTCAACAAATCATAAAGATATTGG
			C1-J-2183	CAACATTTATTTTGATTTTTTGG
			LEPF1	ATTCAACCAATCATAAAGATATTGG
		Reverse	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA
			LEPR1	TAAACTTCTGGATGTCCAAAAAATCA
			C1-N-2191	CCCGGTAAAATTAAAATATAAACTTC
			TL2-N-3014	TCCATTGCACTAATCTGCCATATTA
	CytB	Forward	CytB-Dipt-11035F	GGNTTYKCNGTNGAYAAYGC
			CB-J-10933	GTTTTACCTTGAGGACAAATATC
		Reverse	CytB-Dipt-11545R	ACDGGDCGDGCYCCRATTCA
			TS1-N-11683	AAATTCTATCTTATGTTTTCAAAAC
Nuclear	28S	Forward	rc28AB	ACTACCCCCTGAATTTAAGCA
			F-2	GGATTTTYTKAGTAGCGGCG
		Reverse	28C	GCTATCCTGAGGGAAACTTCGG
	AATS	Forward	1F40	GNATGAAYCARTTYAARCCNAT
			AATS-Dipt-562F	CGNGCHGGHGGHAARCAYAAYGA
			2F	TAYCAYCAYACNTTYTTYGARATG
			AATS-Dipt-631F	ATGYTNGGHAMYTGGTCNTTYGG
		Reverse	1R244	CATNCCRCARTCNATRTGYTT
			AATS-Dipt-840R	GGNCCNVTYTCNCCCATYTCCC
			AATS-Dipt-962R	CGATTRWAYTGWRTRAANACHARRTTCC
	CAD	Forward	CAD-Dipt-757F	AGYAAYGGNCCNGGHGAYCC
			581F2	GGWGGWCAAACWGCWYTMAAYTGYGG
			787F	GGDGTNACNGCNTGYTTYGARCC
			CAD-Dipt-1326F	GGNTCNCARGCNATHAARGC
			CAD-Dipt-1756F	GGNGGNYTNGGNTCNGGNTTYGC
			CAD-Dipt-1911F	TGYATHACNGTNTGYAAYATGG
			CAD-Dipt-2344F	GGHAGYTCNATGAARAGYGTNGG
		Reverse	843R	GCYTTYTGRAANGCYTCYTCRAA
			1098R	TTNGGNAGYTGNCCNCCCAT
			CAD-Dipt-1756R	GCRAANCCNGANCCNARNCCNCC
			CAD-Dipt-2065R	GCRTAYTGDATRTTRCAYTCDCC
			CAD-Dipt-2341R	CCNACRCTYTTCATNGARCTDCC
			CAD-Dipt-3202R	GCRCTRTCDATNGAYTCNGG

2.3. Sequence alignment

Preliminary alignments were produced using ClustalX v2.0.3 (LARKIN et al. 2007) and then examined using Mesquite (MADDISON & MADDISON 2010). Sequences from protein coding genes were checked for stop codons and base call and alignment errors corrected accordingly. 28S preliminary alignments were improved through structural alignment of stem and loop regions following HANCOCK et al. (1988) and KJER et al. (1994), and checked against sequences presented by MENGUAL et al. (2012). The following unconstrained stem and loop regions, based on the numbering of the 28S representation of HANCOCK et al. (1988), were excised due to excessive variation between taxa and an inability to unambiguously align base pairs between them (region of alignment ambiguity): 3749–3774, 3862–3876, 3967–3987, 4133–4135 and 4148–4165.

2.4. Phylogenetic analyses

Molecular datasets. Besides the complete dataset, two subsets were prepared to observe the contribution and effects of each subset on the resulting trees: one with only the protein coding genes (AATS, CAD, COI and CytB) and another with only the protein coding genes minus the 3rd position of each codon. The subsets were made in Mesquite by removing the non-protein coding genes and 3rd codon positions and saving them as a separate files.

Parsimony analyses. All molecular datasets were analyzed separately in TNT v1.1 (GOLOBOFF et al. 2003). All analyses used Traditional Search (Analyze/Traditional Search) with the following parameters: random seed 666, 10,000 replications, TBR with 5 'trees saved per replication', and 'replace existing trees' and 'collapse trees after search' boxes checked. After the analysis, if more than

Table 3. Sequence characteristics. 'Chi square' is the test statistic for homogeneity, 'df' is the degree of freedom of the test (number of taxa – 1 * number of possible bases – 1), and 'P' is the probability of observing a sample statistic as extreme as the 'Chi square'.

	Individual genes						Combined dataset
	12S	**28S**	**AATS**	**CAD**	**COI**	**CytB**	
# characters analysed	359	959	550	1470	1495	715	5548
Constant characters (# / %)	271 / 75.49	770 / 80.29	323 / 58.73	844 / 57.42	901 / 60.27	406 / 56.78	3515 / 63.36
Parsimony-informative characters (# / %)	49 / 13.65	117 / 12.20	201 / 36.54	569 / 38.71	467 / 31.24	246 / 34.40	1649 / 29.72
# parsimony-uninformative characters	39	72	26	57	127	63	384
Average nucleotide frequencies (%)							
A	42.23	35.39	25.43	29.04	31.43	34.47	32.35
C	11.76	14.69	20.48	20.27	13.88	13.14	15.71
G	6.18	18.82	27.45	23.82	14.68	9.89	17.24
T	39.79	31.11	26.64	26.87	40.02	42.50	34.70
Chi-square	8.19	47.94	114.20	124.00	75.41	44.36	567.46
P	1	1	1	0.99	1	1	0
df	177	183	165	162	192	165	192

one parsimonious cladogram was found, a strict consensus cladogram (Nelsen) was generated and saved with the resulting cladograms.

Bayesian and Maximum Likelihood analyses. The GTR+I+G model of character evolution was chosen for all genes through MrModelTest2.3 (Nylander 2004) under the AIC (Akaike Information Criterion) (Posada & Buckley 2004).

Bayesian analyses were run on a parallel version of MrBayes (v.3.2.2) on a computer cluster at the Cyberinfrastructure for Phylogenetic Research (CIPRES) (Miller et al. 2010). Settings and parameters are the same as those used by Gibson et al. (2010a) except the dataset was partitioned into six genes, ngen set to 40,000,000 (sufficient to reach stationarity around generation 32,000,000, i.e., standard deviation split frequencies < 0.0025), burninfrac set to 0.25, printfreq and samplefreq set to 1,000. The final topology is presented as a majority rule consensus tree from all resulting post-burnin trees. Posterior probabilities were viewed in the TreeGraph2 software (Stöver & Müller 2010).

Maximum Likelihood analyses were run on Garli 2.0 (Zwickl 2006) on a computer cluster at CIPRES. Default settings were used except for searchreps = 10, genthreshfortopoterm = 50000 and significanttopochange = 0.001 (based on Mengual et. al. 2015). Trees were viewed, and a majority rule consensus tree was generated, in Mesquite.

2.5. Node support for parsimony analysis

Non-parametric bootstrap (standard) and Jackknife (36% probability of character exclusion) supports were calculated in TNT (Analysis/Resampling) with 10,000 replications, using absolute frequencies. Cut-off was set at 50%. Bremer support was obtained in TNT with the aid of the bremer.run script (included with the TNT software), saving up to 100,000 trees, increasing the score by 1 on each replication until obtaining trees 10 times longer than the shortest one; constrained searches were not performed for groups not lost in suboptimal trees.

3. Results

The final fragment size of the aligned sequences, and the number of parsimony informative sites, is 359 bp (49 informative) for 12S, 550 bp (201 informative) for AATS, 715 bp (246 informative) for CytB, 959 bp (117 informative) for 28S, 1470 bp (569 informative) for CAD, and 1495 bp (569 informative) for COI. There is an A/T bias in all genes varying from 52.07% (AATS) to 82.02% (12S). Base frequencies are homogeneous ($8 < \chi^2 < 125$, $p \geq 0.99$, $162 \leq df \leq 192$) for all taxa on all genes (Table 3). The percentage of characters that are constant varied from 56.78% (CytB) to 80.29% (28S). All sequence characteristics are summarized in Table 3.

The combined dataset has a total of 5548 bp, being 3515 (63.36%) constant and 1649 (29.72%) parsimony informative. Base frequencies revealed an A/T bias (67.05%) and are heterogeneous across all taxa ($\chi^2 > 567.46$, $p < 0.01$, $df = 192$) (Table 3).

3.1. Position of ungrouped species

Previously unplaced taxa had the same stable positions in the resulting trees derived from the different analytical methods (but see sections below). All methods supported the sister species pairing of *O*. cf. *attenuatus* with *O*. aff. *melanorrhinus*, and *O. fascipennis* with *O. lemur*. *Ocyptamus titania* is always associated with the *O. stenogaster* group, and *O. bromleyi*, *O*. cf. *zenillia* and *O*. cf. *zoroaster* always associate with the *O. lepidus* group.

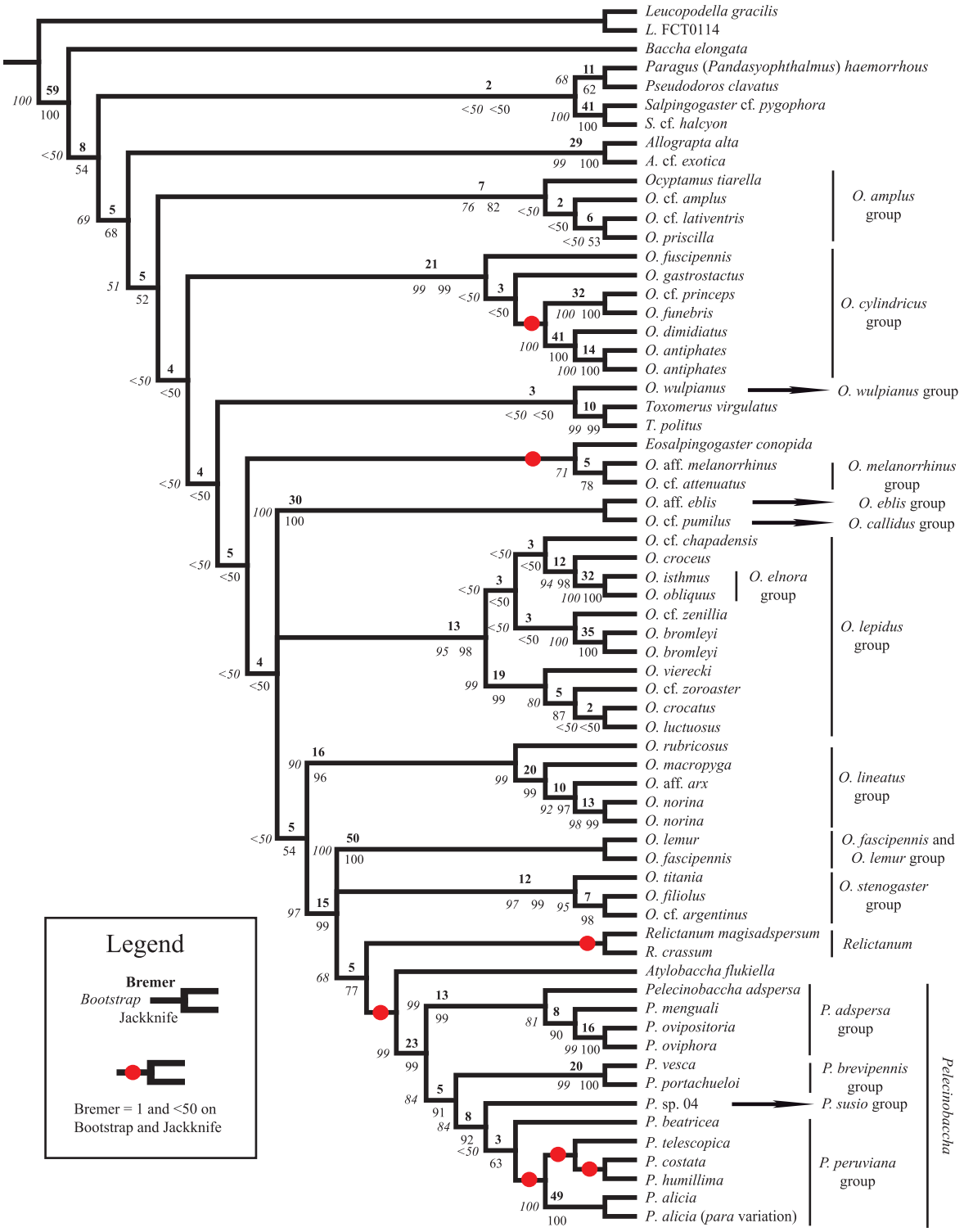

Fig. 1. Strict consensus cladogram (from two most parsimonious cladograms) of the parsimony analysis of the complete dataset. Numbers in bold above branches are Bremer supports, to the left and in italics are bootstrap supports and to the right in normal font are jackknife supports. Red circles indicate branches with Bremer supports = 1 and bootstrap and jackknife supports < 50. Vertical bars and narrow arrows represent genera and species groups recovered in this study.

Ocyptamus cf. *pumilus* was usually recovered with *O.* aff. *eblis* or as sister taxon to a larger lineage. Based on observations of the first author and personal communication with F.C. Thompson, this species belongs to a new group, here referred to as the *O. callidus* group.

3.2. Parsimony analysis

The combined molecular dataset resulted in two most parsimonious cladograms, which are combined into a strict consensus cladogram (Fig. 1, 10753 steps). *Eosal-*

pingogaster conopida and *Toxomerus* are resolved in separate clades among the clades that are part of *Ocyptamus sensu lato*. All other named groups were recovered (Bremer > 10, and Bootstrap and Jackknife > 90), with the exception of *Relictanum* (Bremer = 1, and Bootstrap and Jackknife < 50). Noteworthy results are the *O. elnora* group as part of the *O. lepidus* group, *O. arx* inside the *O. lineatus* group, and the recovery of all species groups of *Pelecinobaccha* as monophyletic.

The analysis of only the protein coding genes generated a similar topology with slightly less support (Electronic Supplement 3). However, some more apical relationships were better supported such as the *Relictanum* and (*Atylobaccha* + *Pelecinobaccha*) clade (Bremer support 3). This analysis resolved the *O. stenogaster* group as sister to (*O. fascipennis* + *O. lemur*) with a Bremer support of 3. The species *P. telescopica* is recovered in a polytomy outside the *P. peruviana* species group in this analysis.

The analysis with only protein coding genes but without the 3rd codon position (Electronic Supplement 4) does not recover many of the groups recovered in the other two analyses, and the ones recovered have less support. *Ocyptamus wulpianus* is placed as the sister taxon to the *O. cylindricus* group, although with low support.

3.3. Bayesian analysis

The Bayesian analysis (Fig. 2) has the *Toxomerus* lineage and *Eosalpingogaster conopida* embedded among the *Ocyptamus* groups, with a posterior probability of 0.98. The same groups recovered in the parsimony analysis are recovered in the Bayesian analysis (posterior probability ≥ 0.99) with the exception of *Relictanum*.

The genus *Relictanum* is not recovered as a natural group, with *R. crassum* sister to (*R. magisadspersum* + (*A. flukiella* + *Pelecinobaccha*)) (0.60). The analysis places *O. wulpianus* in a polytomy with the *O. amplus* group (including *O. tiarella*) and the lineage including the remaining ingroup. The lineage (*O.* cf. *attenuatus* + *O.* aff. *melanorrhinus*), *E. conopida* and the lineage containing the rest of the ingroup are left in a polytomy. *Ocyptamus* aff. *eblis* is placed in a lineage as sister to *O.* cf. *pumilus* (1.00), and this lineage as the sister group to the remaining ingroup (0.84). The ((*O. croceus* + *O. elnora* group) *O. lepidus* group) lineage (1.00) is recovered as the sister group to the remaining ingroup (0.62). The *O. stenogaster* group, including *O. titania*, is resolved as the sister group (1.00) of ((*O. fascipennis* + *O. lemur*) (*Relictanum* (*A. flukiella* + *Pelecinobaccha*))) (0.99). All other relationships found in the parsimony analysis are recovered in the Bayesian analysis with values between 0.74 and 1.00.

The analysis with only the protein coding genes resulted in a similar topology (Electronic Supplement 5), albeit with slightly less support for some groups, plus politomies inside the groups, and the *O. stenogaster* group is resolved as sister to (*O. fascipennis* + *O. lemur*). The analysis with only protein coding genes but without the 3rd codon position is much less resolved with fewer groups being recovered as monophyletic (Electronic Supplement 6), but these few recovered groups agree with groups recovered with the complete dataset.

3.4. Maximum likelihood analysis

The maximum likelihood analysis is the only analysis that resulted in a fully resolved tree (Fig. 3), recovering all named groups as monophyletic, with the exception of *Relictanum*. It has a similar topology to the complete dataset Bayesian analysis, but with different arrangements inside the groups: *Ocyptamus wulpianus* is resolved as the sister taxon to the lineage of *Ocyptamus* groups that contain *Toxomerus* and *E. conopida*, the (*O.* cf. *attenuatus* and *O.* aff. *melanorrhinus*) group is resolved as the sister group to *E. conopida*, and the position of the *O. lepidus* group, including the *O. elnora* group, switches with that of the (*O.* aff. *eblis* + *O.* cf. *pumilus*) lineage.

The analysis with only the protein coding genes resulted in a very similar tree to the one from the complete dataset (Electronic Supplement 7), with a few position changes and one polytomy between four groups. *Ocyptamus wulpianus* is resolved as the sister taxon to *Toxomerus*, and the *O. stenogaster* group is resolved as the sister group to (*O. fascipennis* + *O. lemur*). The analysis with only protein coding genes but without the 3rd codon position (Electronic Supplement 8) is much less resolved than the previous analysis and fewer groups were recovered; this dataset analysis also placed *R. magisadspersum* as sister taxon to the *O. stenogaster* group and *E. conopida* inside *Pelecinobaccha* with 100% support, placements not seen anywhere else in the other analyses.

4. Discussion

4.1. *Ocyptamus* is paraphyletic

The genera *Toxomerus* and *Eosalpingogaster* are resolved among the species of *Ocyptamus*, rendering the latter paraphyletic as observed by Mengual et al. (2008, 2012) and Mengual & Thompson (2011). The genus *Toxomerus* is either recovered as the sister group to *O. wulpianus*, and this lineage sister to the ingroup taxa lineage minus the *O. amplus* and *O. cylindricus* groups (parsimony analysis), or sister to the ingroup taxa lineage minus the groups *O. amplus*, *O. cylindricus* and the species *O. wulpianus* (Bayesian and maximum likelihood analyses). *Eosalpingogaster conopida* is recovered among the ingroup taxa either as sister group to the (*O.* aff. *melanorrhinus* + *O.* cf. *attenuatus*) lineage (parsimony and maximum likelihood analyses) or in a polytomy with the (*O.* aff. *melanorrhinus* + *O.* cf. *attenuatus*) lineage and the remaining ingroup lineage in the Bayesian analysis (Fig. 2).

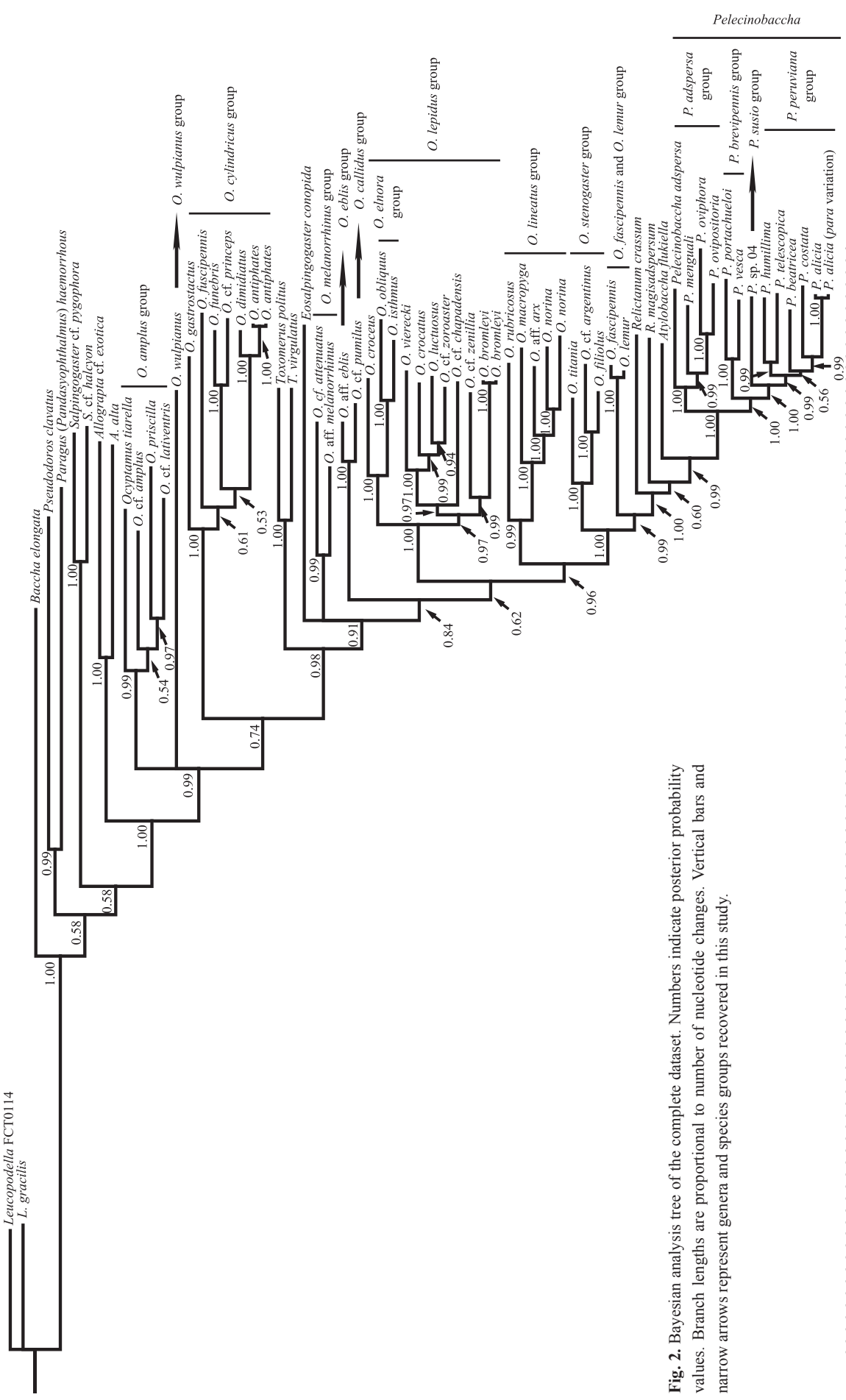

Fig. 2. Bayesian analysis tree of the complete dataset. Numbers indicate posterior probability values. Branch lengths are proportional to number of nucleotide changes. Vertical bars and narrow arrows represent genera and species groups recovered in this study.

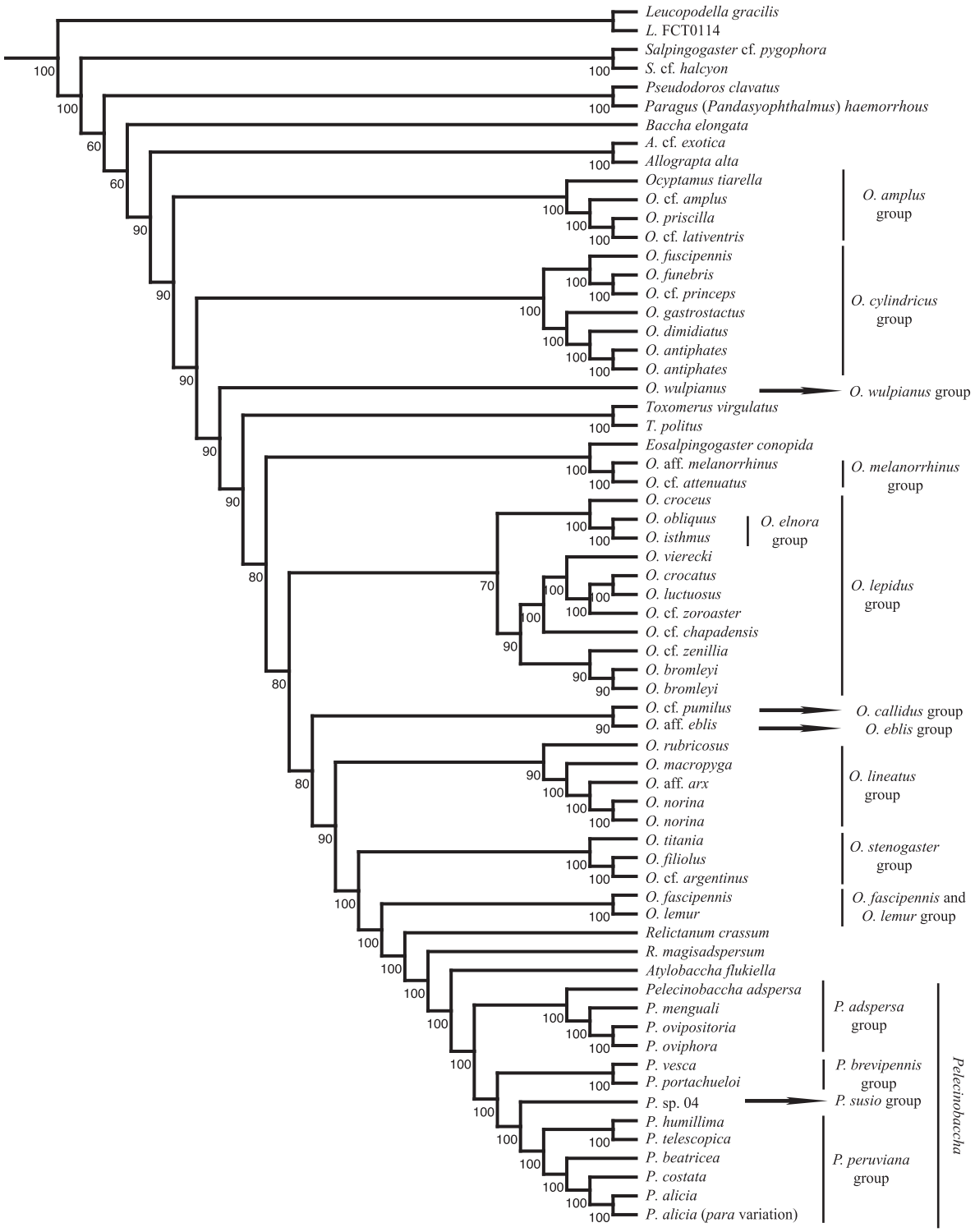

Fig. 3. Maximum likelihood analysis tree of the complete dataset. Numbers below branches indicate percentage of trees in which the branch was recovered. Vertical bars and narrow arrows represent genera and species groups recovered in this study.

Toxomerus has already been reviewed for the West Indies (Thompson 1981) and Brazil (Borges & Couri 2009), and the 'larger species' of the New World (Metz & Thompson 2001). *Toxomerus* is also recovered as a natural group in recent studies (Mengual et al. 2012, 2008). Since the monophyly of *Toxomerus* and its status as a separate genus are not contested, and its placement in the resulting topologies still renders *Ocyptamus* paraphyletic, *Ocyptamus* and its subgroups require redefinition according to phylogenetic principles.

Consistent with Mengual et al. (2008) and Mengual & Thompson (2011), *Eosalpingogaster conopida* is not recovered with other *Salpingogaster* species, further supporting *Eosalpingogaster* as a separate genus and not a subgenus of the latter. Instead, when recovered in a lineage, it is placed as the sister taxon to the (*O.* cf. *attenu-*

atus + *O*. aff. *melanorrhinus*) lineage. *Eosalpingogaster* shows a larval prey preference towards Dactylopiidae (Hemiptera, Sternorrhyncha) (Rojo et al. 2003), unlike any other group studied here. Its placement inside the *Ocyptamus* lineage renders the latter paraphyletic, demonstrating that *Eosalpingogaster* belongs inside the *Ocyptamus sensu lato* lineage, providing yet another argument for redefining *Ocyptamus* and its subgroups.

This study corroborates the hypothesis of Mengual et al. (2012) that *O. fascipennis* and *O. lemur* form a separate group from the *O. cylindricus* species group, and this group is discussed below. The *O. callidus* species group (including *O*. cf. *pumilus*), and the *O. melanorrhinus* species group (including *O*. aff. *melanorrhinus* and O. cf. *attenuatus*) are proposed below. The *O. cylindricus* species group is defined as the only true *Ocyptamus sensu stricto*, and the remaining groups will be formally named, and species assigned, in a future publication (available supraspecific names for the groups are presented in the footnotes below).

Analyses using only the protein coding genes generated similar topologies to those of the complete datasets, with slightly less support to some groups. *Relictanum* has better support, when compared to the complete dataset, in the parsimony analysis. The *O. stenogaster* group plus the (*O. fascipennis* + *O. lemur*) group form a lineage in all three methodologies applied to this dataset. The *O. stenogaster* and (*O. fascipennis* + *O. lemur*) lineage is further supported by biological data, since both groups prey on Pseudococcidae (Table 4) and have a somewhat similar delicate habitus. However, they have different distributions, with the *O. stenogaster* species group being strictly Neotropical and the (*O. fascipennis* + *O. lemur*) group strictly Nearctic.

For this study, the protein coding genes are able to recover many of the relationships seen in the complete dataset, however without as much resolution and support as seen in the complete dataset. The analyses excluding the 3rd codon position still recover several of the groups, but present more polytomies and have less support, which demonstrates the great amount of information enclosed in the 3rd codon position of this sample of taxa.

Some *Ocyptamus* species groups considered in this study show a very specific range of larval prey preferences (Table 4). It is possible that the evolution of these different groups tracked the radiation of their Sternorrhyncha (Hemiptera) prey, which diversified in the late Cretaceous (Grimaldi 2005). Based on a comparison of extant lineages of Diptera basal to Syrphidae, a possible explanation for this radiation would be that specialized predaceous Syrphidae had little competition from other flies for these newly evolving, and thus newly available, lineages of Sternorrhyncha. The only other specialized dipteran predators of Sternorrhyncha at that time (prior to the appearance of predaceous Acalyptratae) would have been a few specialized groups of Cecidomyiinae (Cecidomyiidae). This lack of competition would have facilitated radiation of the syrphines, already made up of Sternorrhyncha predators (e.g. *Allograpta* and *Baccha*), into these niches. Vockeroth (1969) suggests that the great radiation of Syrphini in South America, '*Ocyptamus*' groups among them, took place early in the early Tertiary, following dispersal of ancestors of the group from North America. Although the Panama Isthmus only formed in the Pliocene, there were other landmasses connecting North and South America during the late Paleocene and mid Eocene (Marshall et al. 1997) that would have allowed for the movement of this/these ancestral syrphine(s) into South America. More studies on the life history, group age and larval/pupal morphology will probably facilitate delimitation of the groups presented in this study.

The groups are discussed below in the order they branch out from base to apex of the parsimony cladogram. Available generic names are discussed in the text and included in the footnotes for clarity.

4.2. *Ocyptamus amplus*[1], *globiceps*[2] and *wulpianus*[3] species groups

The *O. amplus* group is either recovered as the sister lineage to the remaining ingroup, including *E. conopida* and the *Toxomerus* lineage (parsimony and maximum likelihood analyses), or in a polytomy with *O. wulpianus* and the remaining ingroup lineage, including *Eosalpingogaster* and *Toxomerus* (Bayesian analysis).

Ocyptamus tiarella (*O. globiceps* (Hull, 1937) group) is recovered as the sister taxon to the *O. amplus* group. Mengual et al. (2012) observed that *O. tiarella* does not belong in the *O. globiceps*, group due to similar placement as observed here, but a broader sample from the *O. globiceps* group, specially *O. globiceps*, would be required to ascertain its relationship with the *O. amplus* group still. *Ocyptamus wulpianus*, when not in a polytomy, is placed as the sister taxon to *Toxomerus* (parsimony analysis) or as sister taxon to the remaining ingroup taxa minus the *O. amplus* and *O. cylindricus* group (maximum likelihood analysis).

The current restricted molecular data (this study only used the 28S and COI data, Electronic Supplements 1 and 2) for *O. wulpianus* and *O. tiarella* is insufficient to confirm the species composition of the *O. amplus* and *O. globiceps* groups. The *O. wulpianus* group is distinguished from the *O. amplus* group species by the linear alula and elongated parallel-sided abdomen with pale fasciae. The *O. globiceps* group is very distinct from the *O. amplus* group since the face and frons are very narrow, the female dorsal occiput has only one row of pile, the scutum is usually distinctly orange to some extent, and the alula is absent. Boyes et al. (1973) had previously distinguished *Orphnabaccha* (= *O. amplus* group) from other "*Baccha*" (= several groups from the current study) by the 2n = 8 karyotype (in contrast to the 2n = 10), which is similar to

[1] = *Orphnabaccha*

[2] = *Pipunculosyrphus*

[3] = *Hermesomyia*

Allograpta Osten Sacken, 1875 and *Pseudoscaeva* (= *O. diversifasciatus* (Knab, 1914) species group, not covered in the current study) karyotypes, which is further evidence for its distinction from the remaining *Ocyptamus* groups.

The *O. amplus* group corresponds to *Orphnabaccha*, established by Hull for *O. coerulea* and synonymized with *Ocyptamus* by Thompson et al. (1976). Hull considered this group to be close to *Ocyptamus* (as *Baccha sensu lato*) but with a pilose metasternum, wide parallel-sided abdomen and with an impressed line on each eye (Hull 1949). Vockeroth (1969) moved more species into *Orphnabaccha*, characterizing the genus by the pilose anterior anepisternum and metasternum, well-developed facial tubercle and absence of pile ventral to the posterior spiracle. Vockeroth also noticed three different male genitalia patterns in the genus (*ampla*, *calda* and *coerulea* types), and commented on the possibility that *Orphnabaccha*, *Hermesomyia* and *Pseudoscaeva* could end up being in one single genus if more species were uncovered, even though each of these genera is very distinct on its own from the others. Thompson (1981) presented the *Oc. caldus* group, added more species to the group, and stated that it was the same as *Orphnabaccha sensu* Hull. *Ocyptamus amplus* group species vary considerably in habitus, with parallel-sided to oval abdomens, and with abdominal markings ranging from strongly patterned (like *Syrphus* Fabricius, 1775) to immaculate with shiny white pile. Most have a pilose metasternum.

The *O. globiceps* group corresponds to the genus *Pipunculosyrphus* erected by Hull (1937), which he based on the distinct characteristics of *P. globiceps* such as the large eyes/head, short abdomen, long wings in comparison to the rest of the body, lack of an alula, narrow face and vertical triangle, and a fringe of pile anteriorly on the scutum. In 1944 (now treating *Pipunculosyrphus* as a subgenus of "*Baccha*") Hull described *P. tiarella* distinguishing it from *P. globiceps* by the connected bands of the abdomen, antero-laterally yellow scutum, yellow scutellum and narrow alula.

A revision of the *O. amplus*, *O. diversifasciatus*, *O. globiceps* and *O. wulpianus* species groups is required to better define the boundaries of these groups.

4.3. *Ocyptamus cylindricus*[4] species group

The *O. cylindricus* group is recovered with strong support (Figs. 1, 2, 3). Species in this group have a pale face, sometimes with a dark, narrow, medial vitta, a brown-pollinose scutum with three inconspicuous vittae of differently oriented pollen, a row of very long shiny pile anteriorly, usually with lateral pale spots anterior to the transverse suture and on the post-alar callus, an elongated and parallel-sided or short and slightly oval abdomen, usually immaculate, mostly dark (at least on basal ½) wings, and a usually greatly elongated subepandrial sclerite. The pedicel medial-apical margin has a narrow extension over the basoflagellomere, unique among the groups studied. The larvae are only known to prey on Aphididae (Hemiptera, Sternorryncha).

This study shows definitively that *O. fascipennis* (not *O. fascipennis* Macquart, 1834 = *O. fuscipennis* (Say, 1823)) and *O. lemur* do not belong to *Ocyptamus sensu stricto* (see redefinition below). Mengual et al. (2012) also supported the monophyly of the *O. cylindricus* group and suggested the removal of *O. fascipennis* from the group.

Hull (1949) based this group on the anterior row of distinct pile on the scutum. Even though he noticed the unique extension of the pedicel margin, he did not emphasize it in the diagnosis or used it in his key. Thompson (1981) improved on the diagnosis of the group but did not mention the pedicel character. The extension of the medial margin of the pedicel is here considered to be both diagnostic and defining for the *O. cylindricus* group. The *O. cylindricus* group includes the type species of the genus and thus should be treated as *Ocyptamus sensu stricto.*

4.4. *Ocyptamus melanorrhinus*[5] species group

The (*O.* cf. *attenuatus* + *O.* aff. *melanorrhinus*) lineage has good support in all analyses (Figs. 1, 2, 3), and is hereby proposed as the *O. melanorrhinus* species group. The two species in this group have a relatively dorsally inserted facial tubercle, an elongated, narrow, parallel-sided abdomen, absent alula and reduced anal lobe. The *O. melanorrhinus* species group is resolved as the sister lineage to *Eosalpinogaster* in the parsimony, although weakly supported, and maximum likelihood analyses. A similar result was obtained by Mengual et al. (2008) with the species *O. melanorrhinus* being placed as the sister taxon to *Eosalpingogaster*, but in Mengual et al. (2012) an undescribed *Ocyptamus* species takes the place of *O. melanorrhinus* as the sister taxon to *Eosalpingogaster* and *O. melanorrhinus* is placed in different positions on the topologies. More species related to this putative group need to be analyzed together to see if it holds up as a natural group related to *Eosalpingogaster*.

4.5. *Ocyptamus eblis*[6] species group

The *O. eblis* group is recovered as the sister taxon to *O.* cf. *pumilus*, and this lineage appears as the sister group to the remaining ingroup (minus the species groups *O. amplus* (including *O. tiarella*), *O. cylindricus*, *O. wulpianus*, *O. melanorrhinus* and the genera *Eosalpingogaster* and *Toxomerus*); in the maximum likelihood analysis this

[4] = *Ocyptamus sensu stricto*

[5] = No current formal group name

[6] = *Styxia*

lineage is at a similar position but apical to the *O. lepidus* group. Species from the *O. eblis* group have a large face and gena, and a spatulate abdomen. *Ocyptamus eblis*, originally *Styxia eblis* (HULL 1943), was distinguished from other *Ocyptamus* species by its pilose eye. A few undescribed species closely resemble *O. eblis* (first author's observations), but have bare eyes instead (as is the case of *O.* aff. *eblis* in this study). Since molecular data were only available from one bare-eyed species (*O.* aff. *eblis*), it remains to be seen if the *O. eblis* group is a natural group. Furthermore, the apparent close relationship between *O.* aff. *eblis* and *O.* cf. *pumilus* might be an artefact of missing data.

4.6. *Ocyptamus callidus*[7] species group

The *O. callidus* species group is proposed for the species *Ocyptamus* cf. *pumilus*. It is recovered as the sister taxon to the *O. eblis* group as mentioned above, although they share no superficial resemblance. It was initially believed, based on its external morphology, that this species was part of the *O. lepidus* group, but it is here treated as a separate species group based on the molecular data. The flies from this group can be readily recognized by the 3 golden pollinose vittae on the scutum, narrow alula, 2nd abdominal segment slightly constricted, pair of 'L'-shaped pale markings on the abdominal tergites and the male's enlarged genitalia.

4.7. *Ocyptamus elnora*[8] and *O. lepidus*[9] species groups

The *O. lepidus* group concept is here expanded to include the representatives of the *O. elnora* group, since the latter is recovered embedded among the former and is supported by all analyses. The lineage made up of *O. croceus* (from the *O. lepidus* group) and the *O. elnora* group is strongly supported in all analyses (Figs. 1, 2, 3), being either the sister group to the remaining *O. lepidus* group (Bayesian and maximum likelihood analyses) or inside the latter (parsimony analysis). The former arrangement seems more likely, since the support for the remaining *O. lepidus* group is very high in the other two analyses compared to the low support observed for the internal arrangement of the *O. lepidus* lineage in the parsimony analysis (Bremer = 3, Bootstrap and Jackknife < 50). *Ocyptamus lepidus* group species have the vertex homogeneously covered by dull white pollen, the scutellum entirely pale, the abdomen slightly petiolate, parallel-sided or spatulate, and the wings entirely light yellow to brown (this diversity of habitus is best seen in the lineage that includes *O. crocatus*, *O.* cf. *zoroaster*, *O. luctuosus* and *O. vierecki*, which is strongly supported in both analyses).

HULL (1949) distinguished the *Baccha lepida* group by the inverted-V pattern on the abdominal tergites, and stated that it was similar to the *B. lineata* group, but still distinguishable, without giving further reasons. THOMPSON (1981) characterizes the *O. lepidus* group and makes the distinction between it and the *O. lineatus* group clear in the diagnosis of the latter. This is the first study that corroborates the monophyly of the *O. lepidus* group.

SHANNON (1927) erected the genus *Calostigma* (= *O. elnora* group) for flies that had a straight M1 vein and an apical dark spot on the wing. THOMPSON (1981) observed that his *O. elnora* group, that he considered to be equivalent to Shannon's *Calostigma*, had two distinct subgroups: "One for those small, mainly yellowish flies that have yellow scutella, and brownish yellow and almost completely microtrichose wings" (group 1) "and another for those larger, mainly black and yellow flies, that have partially black scutella and hyaline and extensively bare wings" (group 2). Representatives of only one of these groups (group 1) were available for this study, but this limited evidence suggests that the *O. elnora* group belongs with the *O. lepidus* group.

4.8. *Ocyptamus arx*[10] and *O. lineatus*[11] species groups

The *O. arx* group is recovered inside the *O. lineatus* group, the former as the sister taxon to *O. norina* (Curran, 1941), in a single lineage with strong support in all analyses. Both groups can be easily identified by the overall pale color, entirely pale face, distinct dull black ocellar triangle amidst the remaining dense white pollen of the vertex/vertical triangle, wings usually yellow tinged on at least the basal ½, petiolate abdomen, and pairs of narrow pale vittae on the abdominal tergites. The species of the *O. lineatus* group have a black scutum covered in dense white pollen with three to four sub-shining vittae, while species of the *O. arx* group have three vittae of golden pollen that are joined together by a circular pollinose area posteriorly. Flies in the *O. arx* group are larger (~15 mm) and have a distinctly petiolate abdomen (narrow 2nd abdominal segment and distinctly widened 3rd and 4th segments).

MENGUAL et al. (2012) also recovered a monophyletic *O. lineatus* group and treated it as the subgenus *O.* (*Hybobathus*), but they did not have a representative from the *O. arx* group (= *Baccha* (*Aulacibaccha*) Hull, 1949) in their analysis. The comment in MENGUAL et al. (2012) about *O. wulpianus* being considered a member of *Aulacibaccha* by HULL (1949) is pertinent since the *pirata* group (where Hull placed *O. wulpianus*) is described under the heading for *Aulacibaccha*. However, Hull stated

[7] = No current formal group name

[8] = *Calostigma*

[9] = No current formal group name

[10] = *Aulacibaccha*

[11] = *Hybobathus*

that "The flies of *Aulacibaccha* contain the *obsoleta* group", which suggests his intent in explicitly naming only that group as part of *Aulacibaccha* and to leave the *pirata* group as just another group of his *Baccha sensu lato*.

Hull (1949) erected the subgenus *Aulacibaccha* for the species with emarginate 4th and 5th abdominal tergites, and mentioned that it holds the largest "*Baccha*" known at the time. He described the distinct ocellar triangle and the abdominal markings of his *Aulacibaccha*, without noting that similar character states occur in the *B. lineata* group, which he instead compared (briefly) to the *B. lepida* and *B. cultrata* groups.

4.9. Species *O. fascipennis* and *O. lemur* [12]

All analyses recovered *O. fascipennis* and *O. lemur* as sister taxa with strong support. Both taxa clearly belong outside *Ocyptamus sensu stricto*. The flies of this group have hyaline wings with a median dark triangular marking, an elongated, narrow, parallel-sided abdomen, and quadrangular or triangular pale maculae baso-laterally on the abdominal tergites. Furthermore, both species are only known to prey on mealybugs (Sternorrhyncha: Coccoidea: Pseudococcidae) (Rojo et al. 2003) and are restricted to the Nearctic region. Our results support the findings of Mengual et al. (2012), who suggested that these species form a group separate from the *O. cylindricus* group. Parsimony analysis places the (*O. fascipennis* + *O. lemur*) lineage in a polytomy with the *O. stenogaster* lineage and the (*Relictanum* (*Atylobaccha* + *Pelecinobaccha*) lineage. Bayesian and maximum likelihood analyses place the (*O. fascipennis* + *O. lemur*) lineage as sister to the lineage containing the genera *Relictanum*, *Atylobaccha* and *Pelecinobaccha* (*sensu* Miranda et al. 2014). The use of more specimens for each group involved in the current study expand on the lineage (*O. stenogaster* + *Pelecinobaccha*) presented by Mengual et al. (2012) by adding more taxa to the lineage, i.e., (*O. stenogaster* ((*O. fascipennis* + *O. lemur*) (*Relictanum* (*Atylobaccha* + *Pelecinobaccha*)))).

4.10. *Ocyptamus stenogaster* species group [13]

The *O. stenogaster* group is always recovered as monophyletic, with *O. titania* as its sister taxon. Distinct characters for the *O. stenogaster* group are the face dark dorsal to the tubercle, tubercle pointed and medially positioned, entirely pale scutellum, an almost complete post-metacoxal bridge, an enlarged epandrium, and a reduced hypandrium. Although *O. titania* differs from the *O. stenogaster* group by several morphological characters, it shares the overall very delicate body (superficially similar to *Baccha* and *Leucopodella*), the lack of an alula, a reduced anal lobe, very long and very narrow abdominal segments, a crescent-shaped 1st abdominal segment with lateral extremities directed laterally, and (usually) quadrangular pale maculae on the baso-lateral corners of the 3rd and 4th abdominal tergites. The morphological similarities between the three species seem to indicate that this arrangement is indeed natural. Mengual et al. (2012) also recognized a lineage made up of *O. stenogaster* and *O.* aff. *stenogaster*, giving further support to this arrangement.

The *O. stenogaster* group was considered by Hull (1949) as *Baccha sensu stricto*, since they were slender to very slender flies, and he divided it in two groups based on black (*obscuricornis* group) or yellow faces (*victoria* group). That author was aware of the variation in the overall color, markings on the abdomen, and presence and shape of the alula, but he did not develop further on the diagnoses. The segmented aedeagus readily separates this group from *Baccha sensu stricto*, which bears an unsegmented aedeagus.

4.11. Genera *Atylobaccha*, *Pelecinobaccha* and *Relictanum*

These genera were reviewed by Miranda et al. (2014). *Pelecinobaccha*, *Relictanum* and *Atylobaccha* are hypothesized to form a monophyletic group. Only the parsimony analysis recovered a *Relictanum* lineage (Bremer support = 1, bootstrap and jackknife < 50) basal to (*Atylobaccha* + *Pelecinobaccha*) (Bremer support = 6, bootstrap and jackknife > 50) with a Bremer support of 5 and bootstrap and jackknife > 50. Larval prey records for this lineage are restricted to the family Coccidae (Hemiptera, Sternorrhyncha) (Table 4), which is hypothesized to be a shift from the Pseudococcidae prey recorded for its two more closely related groups (*O. stenogaster* group and the (*O. fascipennis* + *O. lemur*) group).

Relictanum species and *A. flukiella* are small (6–10 mm) and have a narrow, long 2nd abdominal segment, but *Relictanum* species differ from *A. flukiella* in having a strong facial tubercle and a female cercus covered by setulae (*A. flukiella* has a very weak tubercle and no setulae on the cercus), and both taxa are very distinct from *Pelecinobaccha*. *Atylobaccha* is never recovered within either *Relictanum* or *Pelecinobaccha* in the analyses. Since the Bayesian and maximum likelihood analyses contradict the *Relictanum* lineage of the parsimony analysis, addition of more species of *Relictanum* and *Atylobaccha* are needed in future analyses to test the monophyly of both taxa.

Pelecinobaccha is recovered in both analyses with strong support. The species groups of *Pelecinobaccha* proposed by Miranda et al. (2014) are all recovered with good support in all analyses, with the exception of the *peruviana* group which is recovered with good support in the Bayesian and maximum likelihood analyses but has

[12] = No current formal group name

[13] = No current formal group name

a lower support in the parsimony analysis (Bremer = 3, Bootstrap < 50, Jackknife = 63) (Figs. 1, 2).

5. Conclusions

This study supplements the findings of Mengual et al. (2012) by providing further evidence for the monophyly of the *Ocyptamus cylindricus* and *O. stenogaster* species groups, and for the subgenera *O.* (*Hybobathus*) (= *O. lineatus* group) and *O.* (*Orphnabaccha*) (= *O. amplus* group). Additionally, it corroborates the genus *Pelecinobaccha* as delimited by Miranda et al. (2014). *Ocyptamus lemur* is shown to be the sister taxon to *O. fascipennis*, outside the *O. cylindricus* species group as hypothesized by Mengual et al. (2012). The *O. arx* group was shown to belong within the *O. lineatus* group, and the *O. elnora* group inside the *O. lepidus* group, all monophyletic when these groups are included. The *O. elnora* group needs further study since representatives of only one of its subgroups were available for this study, and this subgroup is clearly part of the *O. lepidus* group. The morphologically distinct *O. callidus*, *O. eblis*, *O. globiceps*, *O. melanorhinus* and *O. wulpianus* species groups are still unresolved, awaiting more specimens or more data to clarify their relationships to the other groups. *Relictanum* is the only taxon not recovered in all analyses, and more taxa are required to test its monophyly.

The genera *Pelecinobaccha*, *Atylobaccha* and *Relictanum*, the *O. stenogaster* species group and the lineage made up of *O. fascipennis* and *O. lemur* together form a monophyletic group with high support in both analyses (Figs. 1, 2). Based on observation of available prey records, the ancestral larvae probably fed on Pseudococcidae with a shift occurring to Coccidae in the (*Relictanum* (*Atylobaccha* + *Pelecinobaccha*)) lineage. It is possible that the range of prey families that *Atylobaccha*, *Pelecinobaccha*, *Relictanum*, the species group *O. stenogaster* and the (*O. fascipennis* + *O. lemur*) lineage attack might be greater than what is apparent, since most of the prey records (all from the superfamily Coccoidea) come only from observation of introduced pests in agricultural crops.

6. Acknowledgements

To F.C. Thompson for inspiration and guidance in the study of the genus *Ocyptamus*. To Ximo Mengual for openly sharing his published and unpublished findings, for helpful discussions on the systematics of Syrphinae, for material loan and comments on a previous version of the manuscript. To Carlos Lamas, Luciane Marinoni, Marcel G. Hermes, Mírian N. Morales and Manuel Zumbado for material loan. To CIPRES Science gateway for the use of its computer clusters for the Bayesian and Maximum Likelihood analyses. To anonymous reviewers for helpful comments on a previous manuscript. Collecting was possible due to Costa Rican permits 048-2008-SINAC and ACG-PI-025-2010 and a Brazilian permit 947-1/10208/SISBIO-IBAMA. Funding was provided to GFGM by Coordenação de Desenvolvimento de Pessoal de Nível Superior (CAPES/ME), to sponsor his PhD studies, and by NSERC funding to JHS and SAM. Funding to JHS by Agriculture and Agri-Food Canada supported molecular work. GFGM is currently funded by the fellowship PCI/INPA/MCTI (process numbers 313083/2013-4 and 313392/2015-3), for studies on Syrphidae.

7. References

Austen E.E. 1893. Description of new species of dipterous insects of the family Syrphidae in the British Museum with notes on species described by the late Francis Walker. – Proceedings of the Zoological Society of London **61**: 131–164.

Borges Z.M., Couri M.S. 2009. Revision of *Toxomerus* Macquart, 1855 (Diptera: Syrphidae) from Brazil with synonymic notes, identification key to the species and description of three new species. – Zootaxa **2179**: 1–72.

Boyes J.W., Boyes B.C., Van Brink J.M., Vockeroth J.R. 1973. Cytotaxonomy of South American Syrphinae (Diptera: Syrphidae). – Genetica **44**: 368–415.

Curran C.H. 1941. New American Syrphidae. – Bulletin of the American Museum of Natural History **78**: 243–304.

Gibson J.F., Skevington J.H., Kelso S. 2010a. Placement of Conopidae (Diptera) within Schizophora based on mtDNA and nrDNA gene regions. – Molecular Phylogenetics and Evolution **56**: 91–103.

Gibson J.F., Kelso S., Skevington J.H. 2010b. Band-cutting no more: A method for the isolation and purification of target PCR bands from multiplex PCR products using new technology. – Molecular Phylogenetics and Evolution **56**: 1126–1128.

Gibson J.F., Kelso S., Jackson M.D., Kits J.H., Miranda G.F.G., Skevington J.H. 2011. Diptera-specific polymerase chain reaction amplification primers of use in molecular phylogenetic research. – Annals of the Entomological Society of America **104**: 976–997.

Goloboff P.A., Farris J., Nixon K. 2003. T.N.T.: Tree Analysis Using New Technology. Version 1.1. – Published by the authors, Tucumán.

Grimaldi D.A. 2005. Evolution of the Insects. – Cambridge University Press, Cambridge. 755 pp.

Hancock J.M., Tautz D., Dover G.A. 1988. Evolution of the secondary structures and compensatory mutations of the ribosomal RNAs of *Drosophila melanogaster*. – Molecular Biology and Evolution **5**: 393–414.

Hull F.M. 1937. New species of exotic syrphid flies. – Psyche **44**: 12–32.

Hull F.M. 1943. The New World species of the genus *Baccha*. – Entomologica Americana **23**: 42–99.

Hull F.M. 1944. Additional species of the genus *Baccha* from the New World. – Bulletin of the Brooklyn Entomological Society **39**: 56–64.

Hull F.M. 1949. The genus *Baccha* from the New World. – Entomologica Americana **27**: 89–291.

Kjer K.M., Baldridge G.D., Fallon A.M. 1994. Mosquito large subunit ribosomal RNA: simultaneous alignment of primary and secondary structure. – Biochimica et Biophysica Acta **1217**: 147–155.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J., Higgins D.G. 2007. Clustal W and Clustal X version 2.0. – Bioinformatics **23**: 2947–2948.

Maddison W.P., Maddison D.R. 2010. Mesquite: a modular system for evolutionary analysis. Version 2.74. – http://mesquiteproject.org.

Marshall S.A. 2012. Flies. The natural history and diversity of flies. – Firefly Books, Buffalo. 616 pp.

Marshall L.G., Sempere T., Butler R.F. 1997. Chronostratigraphy of the mammal-bearing Paleocene of South America. – Journal of the South American Earth Sciences **10**: 49–70.

Mengual X., Thompson F.C. 2011. Carmine cochineal killers: the flower fly genus *Eosalpingogaster* Hull (Diptera: Syrphidae) revised. – Systematic Entomology **36**: 713–731.

Mengual X., Ståhls G., Rojo S. 2008. First phylogeny of predatory flower flies (Diptera, Syrphidae, Syrphinae) using mitochondrial COI and nuclear 28S rRNA genes: conflict and congruence with the current tribal classification. – Cladistics **23**: 1–20.

Mengual X., Ståhls G., Rojo S. 2012. Is the mega-diverse genus *Ocyptamus* (Diptera, Syrphidae) monophyletic? Evidence from molecular characters including the secondary structure of 28S rRNA. – Molecular Phylogenetics and Evolution **62**: 191–205.

Mengual X., Ståhls G., Rojo S. 2015. Phylogenetic relationships and taxonomic ranking of pipizine flower flies (Diptera: Syrphidae) with implications for the evolution of aphidophagy. – Cladistics **31**: 491–508.

Metz M.A., Thompson F.C. 2001. A revision of the larger species of *Toxomerus* (Diptera: Syrphidae) with description of a new species. – Studia Dipterologica **8**: 225–256.

Miller M.A., Pfeiffer W., Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. – Proceedings of the Gateway Computing Environments Workshop (GCE): 1–8.

Miranda G.F.G., Marshall S.A., Skevington J.H. 2014. Revision of the genus *Pelecinobaccha* Shannon, description of *Relictanum* gen. nov. and redescription of *Atylobaccha flukiella* (Curran, 1941). – Zootaxa **3819**: 1–154.

Moulton J.K., Wiegmann B.M. 2004. Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged eremoneuran Diptera (Insecta). – Molecular Phylogenetics and Evolution **31**: 363–378.

Nylander J.A.A. 2004. MrModelTest. Version 2.3. – Evolutionary Biology Centre/Uppsala University, Uppsala.

Posada D., Buckley T. 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike Information Criterion and Bayesian Approaches over Likelihood Ratio Tests. – Systematic Biology **53**: 793–808.

Rojo S., Gilbert F., Marcos-García M.A., Nieto J.M., Mier M.P. 2003. A world review of predatory hoverflies (Diptera, Syrphidae: Syrphinae) and their prey. – CIBIO, Alicante. 319 pp.

Rotheray G.E., Zumbado M.A., Hancock E.G., Thompson F.C. 2000. Remarkable aquatic predators in the genus *Ocyptamus* (Diptera, Syrphidae). – Studia Dipterologica **7**: 385–398.

Shannon R.C. 1927. A review of the South American two-winged flies of the family Syrphidae. – Proceedings of the United States National Museum **70**: 1–34.

Stöver B.C., Müller K.F. 2010. TreeGraph2: Combining and visualizing evidence from different phylogenetic analyses. – BMC Bioinformatics **11**: 7.

Swofford D.L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. – Sinauer, Sunderland.

Thompson F.C. 1981. The flower flies of the West Indies (Diptera: Syrphidae). – Memoirs of the Entomological Society of Washington **9**: 1–200.

Thompson F.C. 1999. A key to the genera of the flower flies (Diptera: Syrphidae) of the Neotropical region including descriptions of new genera and species and a glossary of taxonomic terms. – Contributions on Entomology, International **3**: 321–378.

Thompson F.C., Vockeroth J.R., Sedman Y.S. 1976. Family Syrphidae. Pp. 1–195 in: Papavero N. (ed.), A Catalogue of the Diptera of the Americas South of the United States. – Edanee, São Paulo.

Thompson F.C., Rotheray G.E., Zumbado M.A. 2010. Syrphidae (Flower flies). Pp. 763–792 in: Brown B.V., Borkent A., Cumming J.M., Wood D.M., Woodley N.E., Zumbado M.A. (eds), Manual of Central American Diptera. – NRC Research Press, Ottawa.

Thompson F.C., Zumbado M.A. 2000. Flower flies of the subgenus *Ocyptamus* (*Mimocalla* Hull) (Diptera : Syrphidae). – Proceedings of the Entomological Society of Washington **102**: 773–793.

Ureña O., Hanson P. 2010. A fly larva (Syrphidae: *Ocyptamus*) that preys on adult flies. – Revista de Biología Tropical **58**: 1157–1163.

Vockeroth J.R. 1969. A revision of the genera of the Syrphini (Diptera: Syrphidae). – Memoirs of the Entomological Society of Canada **62**: 1–176.

Zwickl D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation. – The University of Texas, Austin.

Electronic Supplement Files

at http://www.senckenberg.de/arthropod-systematics ("Contents")

File 1: miranda&al-ocyptamus-asp2016-electronicsupplement-1.xls. – Voucher data and GenBank accession numbers for the taxa sampled in this study. '–': Not sequenced.

File 2: miranda&al-ocyptamus-asp2016-electronicsupplement-2.xls. – Tribes, genera and species sampled, and gene fragments successfully obtained in this study.

File 3: miranda&al-ocyptamus-asp2016-electronicsupplement-3.pdf. – Strict consensus cladogram of the parsimony analysis of only the protein coding genes. Numbers in bold above branches are Bremer supports, to the left and in italics are bootstrap supports and to the right in normal font are jackknife supports. Red circles indicate branches with Bremer supports = 1 and bootstrap and jackknife supports < 50. Vertical bars and narrow arrows represent genera and species groups recovered in this study.

File 4: miranda&al-ocyptamus-asp2016-electronicsupplement-4.pdf. – Strict consensus cladogram of the parsimony analysis of only the protein coding genes minus the 3rd codon position. Numbers in bold above branches are Bremer supports, to the left and in italics are bootstrap supports and to the right in normal font are jackknife supports. Red circles indicate branches with Bremer support = 1 and bootstrap and jackknife supports < 50. Vertical bars and narrow arrows represent genera and species groups recovered in this study.

File 5: miranda&al-ocyptamus-asp2016-electronicsupplement-5.pdf. – Bayesian analysis tree of only the protein coding genes. Numbers indicate posterior probability values. Branch lengths are proportional to number of nucleotide changes. Vertical bars and narrow arrows represent genera and species groups recovered in this study.

File 6: miranda&al-ocyptamus-asp2016-electronicsupplement-6.pdf. – Bayesian analysis tree of only the protein coding genes minus the 3rd codon position. Numbers indicate posterior probability values. Branch lengths are proportional to number of nucleotide changes. Vertical bars and narrow arrows represent genera and species groups recovered in this study.

File 7: miranda&al-ocyptamus-asp2016-electronicsupplement-7.pdf. – Maximum likelihood analysis tree of only the protein coding genes. Numbers below branches indicate percentage of trees in which the branch was recovered. Vertical bars and narrow arrows represent genera and species groups recovered in this study.

File 8: miranda&al-ocyptamus-asp2016-electronicsupplement-8.pdf. – Maximum likelihood analysis tree of only the protein coding genes minus the 3rd codon position. Numbers below branches indicate percentage of trees in which the branch was recovered. Vertical bars and narrow arrows represent genera and species groups recovered in this study.