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# Molecular phylogeny of *Allograpta* (Diptera, Syrphidae) reveals diversity of lineages and non-monophyly of phytophagous taxa

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ABSTRACT

Phylogenetic relationships of genera *Allograpta*, *Sphaerophoria* and *Exallandra* (Diptera, Syrphidae) were analyzed based on sequence data from the mitochondrial protein-coding gene cytochrome *c* oxidase subunit I (COI) and the nuclear 28S and 18S ribosomal RNA genes. The three genera are members of the subfamily Syrphinae, where nearly all members feed as larvae on soft-bodied Hemiptera and other arthropods. Phytophagous species have recently been discovered in two subgenera of *Allograpta*, sg *Fazia* and a new subgenus from Costa Rica. Phylogenetic analyses of the combined datasets were performed using parsimony, under static alignment and direct optimization, maximum likelihood and Bayesian inference. Congruent topologies obtained from all the analyses indicate paraphyly of the genus *Allograpta* with respect to *Sphaerophoria* and *Exallandra*. *Exallandra* appears embedded in the genus *Sphaerophoria*, and both genera are placed within *Allograpta*. The distribution of phytophagous taxa in *Allograpta* indicates that plant feeding evolved at least twice in this group.



## 1. Introduction

The family Syrphidae (hover or flower flies) comprises almost 6000 described species and is nearly worldwide in distribution (Thompson, 2006). Current classification of Syrphidae recognizes three subfamilies, Microdontinae, Eristalinae and Syrphinae, and 14 tribes in total (Thompson and Rotheray, 1998). Syrphids are typically black with yellow and orange markings, particularly visible on abdomen. This coloration of many species causes them to be confused with bees or wasps; they are excellent mimics of aculeate Hymenoptera. The adults of the family have the ability to hover motionless in the air and are associated with flowers, which are used as energy sources (pollen and nectar) and as mating sites. Syrphid larvae are very variable in structure, habits and feeding modes. All known microdontines are ant brood predators, while the subfamily Eristalinae includes feeding modes as diverse as saprophagy, phytophagy, mycophagy, and, immatures of the genus *Volucella* which are wasp and bee brood predators (Rotheray, 1993; Rotheray and Gilbert, 1999). The members of the eristaline tribe Pipizini are aphid predators, sharing this feeding mode with the subfamily Syrphinae.

Members of the subfamily Syrphinae have larvae that are predacious mostly on soft-bodied Hemiptera, but includes also taxa that prey on larvae of Neuroptera, other Diptera, Acari, Lepidoptera, Coleoptera or Thysanoptera (for a review see Rojo et al., 2003). Hamrum (1966) concluded that the feeding habits of common Syrphinae species probably range from obligate aphid feeders (e.g. some *Eupeodes*) to facultative phytophagous–aphidophagous forms as *Melanostoma* spp. to primarily phytophagous species like *Toxomerus politus*, "the corn-feeding syrphid fly". Literature records on presumed or more well-documented phytophagy of *Toxomerus politus* larvae includes Riley and Howard (1888), Richardson (1915), Rosewall (1916), and Smith (1974), reporting observations of pollen feeding and feeding on the sap from the saccharine cells of *Zea mays*. Some taxa of genera *Melanostoma* and *Platycheirus* (tribe Bacchini) have been shown to facultatively feed on decaying vegetal matter (Goeldlin de Tiefenau, 1974; Rotheray and Gilbert, 1989).

### 1.1. Allograpta Osten Sacken, 1875

Genus *Allograpta*, member of the subfamily Syrphinae, was established for *Scaeva obliqua* Say, 1823 described from North America (USA). *Allograpta* comprises species of great morphological variation, very slender to moderately robust (length 5.9–

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13.7 mm), with different patterns of yellow markings on abdomen (oblique markings, roundish spots or stripes), and with lower part of face either receding, produced forwards or downwards. Vockeroth (1969, 1973a) defined the current concept of *Allograpta* and discussed the great variation in size, body shape, colour pattern, and head shape within the genus. He was the first to recognize that Old World species belonged to the genus described from the New World. *Allograpta* flower flies are distributed almost worldwide, except not known from the northern areas of the Nearctic region and in the Palaearctic region distributed only in its south-eastern portion (Vockeroth, 1969).

The total number of described species reaches 106 divided in six subgenera (from Thompson, 2006; Mengual et al., unpublished; summarized in Table 1). Highest diversity of species and subgenera is found in Neotropics, with 56 species and all six subgenera present. The subgenera are *Allograpta* s. str. Osten Sacken, 1875, *Antillus* (Vockeroth, 1969), *Claraplumula* (Shannon, 1927), *Fazia* (Shannon, 1927), *Rhinoprosopa* (Hull, 1942), and a new subgenus found only in Costa Rica referenced here as subgenus CR, described by Mengual et al. (unpublished). All these subgenera have had generic status in the past, but with a described close relationship with *Allograpta*. Consequently, Neotropical specimens are the most abundant in our study. Only the subgenus *Allograpta*, rather uniform except Australian and New Zealander species, is distributed outside the Neotropical region.

A first indication of genus *Allograpta* as being not obligatory predatory was Davidson (1922) observation of larvae of *Allograpta obliqua* being able to sustain themselves on plant food. The significant finding of Nishida et al. (2002) reported the first clear case in which a reared syrphine species, *Allograpta centropogonis* Nishida, 2002, was shown to feed on live plant tissue, mining the leaves of four species of *Centropogon* (Campanulaceae). This species belongs to subgenus *Fazia* and there are three additional undescribed species differing only in the details of the male genitalia (Nishida et al., 2002). Recently, another non-predacious *Allograpta* (classified into the same subgenus) was discovered at high elevations in Costa Rica when the larvae of *A. (Fazia) micrura* (Osten Sacken, 1877) were found to feed on pollen of *Castilleja* (Scrophulariaceae) flowers (Weng and Rotheray, unpublished).

Van Zuijen and Nishida (unpublished) found *Allograpta zumbadoi* Thompson, 2000 boring the stems of *Centropogon* plants. This species is placed in a new subgenus (Mengual et al., unpublished) as is another new phytophagous species described in this world revision. Both species are leaf miners in early stages and stem borers in late stages on several *Centropogon* species (Van Zuijen and Nishida, unpublished).

Despite the great variation within the genus, Vockeroth (1969, p. 128) listed four morphological characters that distinguish *Allograpta* species from all other Syrphinae genera except *Sphaerophoria* and *Exallandra*, and indicated the difficulty to distinguish *Sphaerophoria* [and *Exallandra*] females from those of some species of the subgenus *Allograpta*, "specifically if the latter lack the oblique tergite markings commonly found in that genus". Vockeroth noted differences in males, from *Sphaerophoria* (smaller genitalia) and from *Toxomerus* [as *Mesograpta*] (male vertical triangle and mesonotal coloration).

Fluke (1929) and Heiss (1938) mentioned the morphological similarity between the larva of *Sphaerophoria* and *Allograpta*. The characters given by Dušek and Láska (1967) suggested that the larvae of *Sphaerophoria* are very similar to those of *Episyrphus*. Shatalkin (1975), however, concluded that *Allograpta* and *Sphaerophoria* are closely connected with the tribe Bacchini. In their 1999 study, Rotheray and Gilbert recovered a phylogenetic tree based on larval characters with a clade formed by *Baccha* as sister group to *Allograpta* and *Sphaerophoria*. More recently, Mengual et al. (2008) presented a preliminary molecular phylogeny of Syrphinae and concluded that the genus *Allograpta* is paraphyletic with respect to *Sphaerophoria*, but with no close relationship with *Baccha*. Thus, *Allograpta* and *Sphaerophoria* were shown to be closely related based on separate studies on adult morphology, immature characters and DNA sequences. Results of Mengual et al. (2008) resolved *Allograpta* + *Sphaerophoria* as sister group to genera ((*Episyrphus* + *Meliscaeva*) + *Asarkina*). As a sister group of this grouping were placed some genera like *Toxomerus*, *Ocyptamus*, *Paragus* and *Allobaccha*.

### 1.2. Aims of the study

To explore the monophyly of and the relationships between the subgenera of *Allograpta* are the major aims of this study. We also attempted to anwser if phytophagy has evolved several times within the group. Additionally, we were interested to re-address the phylogenetic relationships between *Allograpta*, *Sphaerophoria* and *Exallandra* found by Mengual et al. (2008). Thus, a fragment of 18S rRNA gene (approx. 350 nucleotides) was used, in addition to the mitochondrial protein-coding gene cytochrome *c* oxidase subunit I (COI) and the nuclear D2-3 28S previously used by Mengual et al. (2008). Especially the two latter gene regions have proved to be informative for species-level and genus-level analyses, as demonstrated in a large number of studies of insect evolutionary relationships (e.g. Caterino et al., 2001; Ståhls et al., 2003, 2004; Arevalo et al., 2004; Kjer, 2004; Mengual et al., 2006; Ståhls, 2006; Brammer and von Dohlen, 2007; Milankov et al., 2007).

## 2. Materials and methods

### 2.1. Taxon sampling

The taxon sampling was done trying to cover as much taxonomic diversity as possible, particularly of the genera *Allograpta* and *Sphaerophoria*. We included representatives of five subgenera of *Allograpta* (specimens of the sixth sg. *Claraplumula* could not be obtained for molecular analysis), and most included species have Neotropical or Afrotropical distributions (see Table 1). Finally, 37 specimens of the genus *Allograpta* were included in the analysis representing 34 putative species (32.1% of known species) (Table 2). The highest diversity in number of species and subgenera is found in the Neotropical region, and it was represented in our study by 33 Neotropical specimens, and two specimens from Nearctic and two additional from Afrotropical region (see Table 1). In a subgeneric classification view, 20 specimens of *Fazia*, four species of *Rhinoprosopa*, one species of the new subgenus CR (*Allograpta zumbadoi*) and one of *Antillus* (the unique described species of these two last subgenera), and 11 specimens of the subgenus *Allograpta* were included in the analyses (Table 2).

We also included six species of the genus *Sphaerophoria* (out of 75; Thompson, 2006) and one representative of the Afrotropical genus *Exallandra* (monotypic genus with *E. cinctifacies*). *Ocyptamus wulpianus* was chosen as outgroup, based on the results of Mengual et al. (2008), and we additionally included multiple representatives of the genera *Asarkina*, *Meliscaeva* and *Episyrphus*, *Salpingogaster* s. str. + subgenus *Eosalpingogaster*, *Ocyptamus* and *Toxomerus*. When possible, more than one specimen of the same species was included to test if they were recovered together in the resulting phylogenetic trees. Nomenclature follows mainly the Biosystematic Database of World Diptera for species names (Thompson, 2006). An identification key for *Allograpta* (Thompson, manuscript) was used, and doubtful identifications were checked by F.C. Thompson, Systematic Entomology Laboratory, US Department of Agriculture, Smithsonian Institution. Unnamed taxa, referred as sp. 1, sp. 2, etc., are with high probability species new to science. Species new to science identified by F.C. Thompson (*in litt.*) from Costa Rica are de-

**Table 1**
Attempted synthesis of described species names in the genus *Allograpta*, based on The Biosystematic Database of World Diptera (Thompson, 2006)

| Neotropical | Neotropical |
|---|---|
| *Allograpta* (*Allograpta*) *aeruginosifrons* (Schiner, 1868) | *Allograpta* (*Fazia*) *syrphica* (Giglio-Tos, 1892) |
| *Allograpta* (*Allograpta*) *annulipes* (Macquart, 1850) | *Allograpta* (*Antillus*) *ascita* (Vockeroth (1969)) |
| *Allograpta* (*Allograpta*) *aperta* Fluke, 1942 | *Allograpta* (*Claraplumula*) *latifacies* (Shannon, 1927) |
| *Allograpta* (*Allograpta*) *bilineella* Enderlein, 1938 | *Allograpta* (CR) *zumbadoi* Thompson, 2000 |
| *Allograpta* (*Allograpta*) *browni* Fluke, 1942 | *Allograpta* (*Fazia*) *alta* Curran, 1936 |
| *Allograpta* (*Allograpta*) *colombia* Curran, 1925 | *Allograpta* (*Fazia*) *altissima* (Fluke, 1942) |
| *Allograpta* (*Allograpta*) *exotica* (Wiedemann, 1830) | *Allograpta* (*Fazia*) *argentipila* (Fluke, 1942) |
| *Allograpta* (*Allograpta*) *falcata* Fluke, 1942 | *Allograpta* (*Fazia*) *centropogonis* Nishida, 2002 |
| *Allograpta* (*Allograpta*) *hastata* Fluke, 1942 | *Allograpta* (*Fazia*) *decemmaculata* (Rondani, 1863) |
| *Allograpta* (*Allograpta*) *hortensis* (Pjilippi, 1865) | *Allograpta* (*Fazia*) *eupeltata* Bigot, 1884 |
| *Allograpta* (*Allograpta*) *insularis* Thompson, 1981 | *Allograpta* (*Fazia*) *fasciata* Curran, 1932 |
| *Allograpta* (*Allograpta*) *limbata* (Fabricius, 1805) | *Allograpta* (*Fazia*) *fascifrons* Macquart, 1846 |
| *Allograpta* (*Allograpta*) *mu* (Bigot, 1884) | *Allograpta* (*Fazia*) *flukei* Curran, 1936 |
| *Allograpta* (*Allograpta*) *neosplendens* Thompson, in litt. | *Allograpta* (*Fazia*) *forreri* (Giglio-Tos, 1893) |
| *Allograpta* (*Allograpta*) *neotropica* Curran, 1936 | *Allograpta* (*Fazia*) *funeralia* (Hull, 1944) |
| *Allograpta* (*Allograpta*) *obliqua* (Say, 1823) | *Allograpta* (*Fazia*) *hians* (Enderlein, 1938) |
| *Allograpta* (*Allograpta*) *piurana* Shannon, 1927 | *Allograpta* (*Fazia*) *imitator* (Curran, 1925) |
| *Allograpta* (*Allograpta*) *pulchra* Shannon, 1927 | *Allograpta* (*Fazia*) *luna* (Fluke, 1942) |
| *Allograpta* (*Allograpta*) *quadricincta* Enderlein, 1938 | *Allograpta* (*Fazia*) *macquarti* (Blanchard, 1852) |
| *Allograpta* (*Allograpta*) *radiata* (Bigot, 1857) | *Allograpta* (*Fazia*) *micrura* (Osten Sacken, 1877) |
| *Allograpta* (*Allograpta*) *robinsoniana* Enderlein, 1938 | *Allograpta* (*Fazia*) *nasigera* (Enderlein, 1938) |
| *Allograpta* (*Allograpta*) *tectiforma* Fluke, 1942 | *Allograpta* (*Fazia*) *plaumanni* (Frey, 1946) |
| *Allograpta* (*Allograpta*) *teligera* Fluke, 1942 | *Allograpta* (*Fazia*) *remigis* (Fluke, 1942) |
| *Allograpta* (*Fazia*) *roburoris* (Fluke, 1942) | *Allograpta* (*Rhinoprosopa*) *aenea* (Hull, 1937) |
| *Allograpta* (*Fazia*) *rostrata* (Bigot, 1884) | *Allograpta* (*Rhinoprosopa*) *flavophylla* (Hull, 1943) |
| *Allograpta* (*Fazia*) *saussurii* (Giglio-Tos, 1892) | *Allograpta* (*Rhinoprosopa*) *lucifera* (Hull, 1943) |
| *Allograpta* (*Fazia*) *similis* Curran, 1925 | *Allograpta* (*Rhinoprosopa*) *neonasuta* Thompson, in litt. |
| *Allograpta* (*Fazia*) *strigifacies* (Enderlein, 1938) | *Allograpta* (*Rhinoprosopa*) *sycorax* (Hull, 1947) |
| **Nearctic** | **Oriental** |
| *Allograpta* (*Allograpta*) *exotica* (Wiedemann, 1830) | *Allograpta* (*Allograpta*) *dravida* Ghorpade, 1994 |
| *Allograpta* (*Allograpta*) *obliqua* (Say, 1823) | *Allograpta* (*Allograpta*) *javana* (Wiedemann, 1824) |
| *Allograpta* (*Allograpta*) *radiata* (Bigot, 1857) | *Allograpta* (*Allograpta*) *kinabalensis* (Curran, 1931) |
| *Allograpta* (*Fazia*) *forreri* (Giglio-Tos, 1893) | *Allograpta* (*Allograpta*) *maculipleura* (Brunetti, 1913) |
| *Allograpta* (*Fazia*) *micrura* (Osten Sacken, 1877) | *Allograpta* (*Allograpta*) *medanensis* (Meijere, 1914) |
| | *Allograpta* (*Allograpta*) *obscuricornis* (Meijere, 1914) |
| **Palaearctic** | *Allograpta* (*Allograpta*) *philippina* (Frey, 1946) |
| *Allograpta* (*Allograpta*) *javana* (Wiedemann, 1824) | *Allograpta* (*Allograpta*) *purpureicollis* (Frey, 1946) |
| *Allograpta* (*Allograpta*) *maritima* Mutin, 1986 | *Allograpta* (*Allograpta*) *robinsoni* (Curran, 1928) |
| **Afrotropical** | **Australasian** |
| *Allograpta* (*Allograpta*) *borbonica* Kassebeer, 2000 | *Allograpta* (*Allograpta*) *alamacula* Carver, 2003 |
| *Allograpta* (*Allograpta*) *calopoides* (Curran, 1938) | *Allograpta* (*Allograpta*) *amphoterum* (Bezzi, 1928) |
| *Allograpta* (*Allograpta*) *calopus* (Loew, 1858) | *Allograpta* (*Allograpta*) *atkinsoni* (Miller, 1921) |
| *Allograpta* (*Allograpta*) *fuscotibialis* (Macquart, 1842) | *Allograpta* (*Allograpta*) *buruensis* Meijere, 1929 |
| *Allograpta* (*Allograpta*) *hypoxantha* (Bezzi, 1923) | *Allograpta* (*Allograpta*) *australensis* (Schiner, 1868) |
| *Allograpta* (*Allograpta*) *nasuta* (Macquart, 1842) | *Allograpta* (*Allograpta*) *citronella* (Shiraki, 1963) |
| *Allograpta* (*Allograpta*) *nummularia* (Bezzi, 1920) | *Allograpta* (*Allograpta*) *dorsalis* (Miller, 1924) |
| *Allograpta* (*Allograpta*) *phaeoptera* (Bezzi, 1920) | *Allograpta* (*Allograpta*) *exotica* (Wiedemann, 1830)* |
| *Allograpta* (*Allograpta*) *rediviva* (Bezzi, 1915) | *Allograpta* (*Allograpta*) *flavofaciens* (Miller, 1921) |
| *Allograpta* (*Allograpta*) *rufifacies* (Keiser, 1971) | *Allograpta* (*Allograpta*) *hirsutifera* (Hull, 1949) |
| *Allograpta* (*Allograpta*) *tenella* (Keiser, 1971) | *Allograpta* (*Allograpta*) *hudsoni* (Miller, 1921) |
| *Allograpta* (*Allograpta*) *varipes* (Curran, 1927) | *Allograpta* (*Allograpta*) *javana* (Wiedemann, 1824) |
| | *Allograpta* (*Allograpta*) *longulus* (Shiraki, 1963) |
| | *Allograpta* (*Allograpta*) *neofasciata* Thompson, 1989 |
| | *Allograpta* (*Allograpta*) *nigripilosa* (Hull, 1944) |
| | *Allograpta* (*Allograpta*) *obliqua* (Say, 1823)* |
| | *Allograpta* (*Allograpta*) *pallida* (Bigot, 1884) |
| | *Allograpta* (*Allograpta*) *pseudoropalus* (Miller, 1921) |
| | *Allograpta* (*Allograpta*) *pulchra* Shannon, 1927** |
| | *Allograpta* (*Allograpta*) *radiata* (Bigot, 1857)* |
| | *Allograpta* (*Allograpta*) *ropalus* (Walker, 1849) |
| | *Allograpta* (*Allograpta*) *septemvittata* (Shiraki, 1963) |
| | *Allograpta* (*Allograpta*) *ventralis* (Miller, 1921) |

| Subgenus | Number of species analyzed | Total number of species |
|---|---|---|
| *Allograpta* | 10 | 73 |
| *Antillus* | 1 | 1 |
| *Claraplumula* | 0 | 1 |
| New subgenus *CR* | 1 | 1 |
| *Fazia* | 18 | 25 |
| *Rhinoprosopa* | 4 | 5 |

Summary of analyzed versus total number of species by subgenus.
* Introduced species only found in Hawaii from Australasian region.
** Species only found in Easter Island from Australasian region.

**Table 2**
MZH voucher codes, general locality information, and GenBank accession numbers for taxa used in this study

| Taxon | MZH voucher | Label information | Accession No. COI | Accession No. 28S | Accesion No. 18S |
|---|---|---|---|---|---|
| *Allograpta* (*Allograpta*) aff. *exotica* | XP193 | 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.: G. Ståhls. Det.: X. Mengual. | EU241708 | EU241756 | EU241804 |
| *Allograpta* (*Allograpta*) *calopus* (Loew, 1858) | XP39 | SOUTH AFRICA, Western Cape, Constantia. 34°092' 24"S 18°25'43"E. 260 m. 28-IX/06-X-2004. Leg.: M.E. Irwin, F. Parker & M. Hauser. Det.: F.C. Thompson. | EF127311 | EF127390 | |
| *Allograpta* (*Allograpta*) *exotica* (Wiedemann, 1830) | XP35 | USA, New Mexico, Chaves Sagebrush Valley road at Squa Canyon riad. 32° 57'N 104° 50'W. 1/10-V-2004. Leg.: M.E. Irwin & F. Parker. Det.: F.C. Thompson. | EF127308 | EF127387 | |
| *Allograpta* (*Allograpta*) *falcata* Fluke, 1942 | XP63 | COLOMBIA, Dpto Caldas, Villamaría. Via La Esperanza km 8. 2530 m. 19-II-2006. Leg.: X. Mengual. Det.: X. Mengual. | EU241709 | EU241757 | EU241805 |
| *Allograpta* (*Allograpta*) *fuscotibialis* (Macquart, 1842) | XP37 | SOUTH AFRICA, Western Cape, Constantia. 34°092' 24"S 18°25'43"E. 260 m. 28-IX/06-X-2004. Leg.: M.E. Irwin, F. Parker & M. Hauser. Det.: F.C. Thompson. | EF127309 | EF127388 | |
| *Allograpta* (*Allograpta*) *hastata* Fluke, 1942 | XP61 | COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 15-II-2006. 2175 m. Leg.: X. Mengual. Det.: X. Mengual. | EU241710 | EU241758 | EU241807 |
| *Allograpta* (*Allograpta*) *neotropica* Curran, 1936 | XP59 | COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 15-II-2006. 2175 m. Leg.: X. Mengual. Det.: X. Mengual. | EU241733 | EU241780 | EU241831 |
| *Allograpta* (*Allograpta*) *neotropica* Curran, 1936 | XP62 | COLOMBIA, Dpto Valle del Cauca. Cali, km18. Cerro San Antonio. 2175 m. (radiotowers) 24-II-2006. 0329.377'N 76°33.495'W. Leg.: X. Mengual. Det.: X. Mengual. | EU241734 | EU241781 | EU241832 |
| *Allograpta* (*Allograpta*) *obliqua* (Say, 1823) | 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* (*Allograpta*) sp. 1 | XP64 | COLOMBIA, Dpto Valle del Cauca. Cali, Corrg. Los Andes. Pichindé, El Faro. 1700 m. 15-II-2006. Leg.: X. Mengual. Det.: X. Mengual. | EU241711 | EU241759 | EU241808 |
| *Allograpta* (*Allograpta*) *teligera* Fluke, 1942 | XP149 | COSTA RICA, P.N. Tapantí, Estación "La Esperanza", 2600 m. 13-I-2005. Leg.: F.C. Thompson. Det.: F.C. Thompson. | EU241712 | EU241760 | EU241809 |
| *Allograpta* (*Antillus*) *ascita* (Vockeroth (1969)) | 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* (CR) *zumbadoi* Thompson, 2000 | 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.: K. Nishida | EU241714 | EU241762 | EU241811 |
| *Allograpta* (*Fazia*) aff. *centropogonis* | XP151 | COSTA RICA, INBio code: 3430. Det.: INBio staff. | EU241715 | EU241763 | EU241812 |
| *Allograpta* (*Fazia*) aff. *fasciata* | XP81 | COSTA RICA, P.N. Tapantí. Estación La Esperanza. 13-I-2005. 2800 m. Det.: X. Mengual. | EU241716 | EU241764 | EU241813 |
| *Allograpta* (*Fazia*) aff. *imitator* | XP57 | 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. | EU241717 | EU241765 | EU241814 |
| *Allograpta* (*Fazia*) *centropogonis* Nishida, 2003 | 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*) cf. *luna* | XP72 | COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 15-II-2006. 2200 m. Leg.: X. Mengual. Det.: F.C. Thompson. | EU241718 | EU241766 | EU241815 |
| *Allograpta* (*Fazia*) *CR-5* Thompson, *in litt.* | XP205 | 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.: F.C. Thompson. | EU241719 | EU241767 | EU241817 |
| *Allograpta* (*Fazia*) *CR-7* Thompson, *in litt.* | XP56 | COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 15-II-2006. 2200 m. Leg.: X. Mengual. Det.: X. Mengual. | EU241720 | EU241768 | EU241818 |
| *Allograpta* (*Fazia*) *fasciata* Curran, 1932 | S490 | COSTA RICA, P.N. Tapantí, 1600 m., 12-I-2005. Leg.: C. Pérez-Bañón. Det.: F.C. Thompson. | EF127366 | EF127445 | EU241806 |
| *Allograpta* (*Fazia*) *fascifrons* (Macquart, 1846) | XP60 | COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 15-II-2006. 2200 m. Leg.: X. Mengual. Det.: F.C. Thompson. | EU241736 | EU241783 | EU241835 |
| *Allograpta* (*Fazia*) *fascifrons* (Macquart, 1846) | XP69 | COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 15-II-2006. 2200 m. Leg.: C. Gutiérrez. Det.: F.C. Thompson. | EU241737 | EU241784 | EU241836 |
| *Allograpta* (*Fazia*) *fascifrons* (Macquart, 1846) | XP25 | COLOMBIA, Cali, Cerro San Antonio. 2180 m. 20-VII-2004. Leg.: C. Prieto. Det.: F.C. Thompson. | EF127300 | EF127379 | |
| *Allograpta* (*Fazia*) *hians* (Enderlein, 1938) | XP171 | VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. 25-I-2007. N10° 20.828' W067° 41.309'. Leg.: X. Mengual. Det.: F.C. Thompson. | EU241721 | EU241769 | EU241819 |
| *Allograpta* (Fazia) imitator (Curran, 1925) | XP156 | VENEZUELA, Edo. Aragua. P.N. Henri Pittier, Portachuelo, 1152 m. N10° 20.828' W067° 41.309'. 26-I-2007. Leg.: X. Mengual. Det.: F.C. Thompson. | EU241722 | EU241770 | EU241820 |
| *Allograpta* (*Fazia*) *micrura* (Osten Sacken, 1877) | 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* (*Fazia*) *roburoris* Fluke, 1942 | XP181 | VENEZUELA, Edo. Miranda. San Antonio de los Altos, IVIC. 01-02-2007. N10° 24.069' W066° 58.667'. Leg.: X. Mengual. Det.: X. Mengual. | EU241735 | EU241782 | EU241834 |
| *Allograpta* (*Fazia*) *rostrata* (Bigot, 1884) | XP66 | COLOMBIA, Dpto Valle del Cauca. Palmira, Corrg. La Buitrera. Nirvana. 14-II-2006. 1560-1600 m. Leg.: X. Mengual. Det.: X. Mengual. | EU241724 | EU241772 | EU241822 |
| *Allograpta* (*Fazia*) sp. 2 | XP150 | COSTA RICA, INBio code: 3411. Det.: INBio staff. | EU241725 | EU241773 | EU241823 |
| *Allograpta* (*Fazia*) sp. 3 | XP143 | MEXICO, Veracruz, Coatepec. 28-X-2006. Leg.: V. Vahtera. Det.: X. Mengual. | EU241726 | EU241774 | EU241824 |

| | | | | | |
|---|---|---|---|---|---|
| *Allograpta* (*Fazia*) sp. 4 | S491 | COSTA RICA, P.N. Tapantí, 1600 m., 12-I-2005. Leg.: M. Zumbado. Det.: X. Mengual. | EU241727 | EF127446 | EU241825 |
| *Allograpta* (*Fazia*) strigifacies (Enderlein, 1938) | XP67 | COLOMBIA, Dpto Valle del Cauca. Cali, Cerro San Antonio. 15-II-2006. 2200 m. Leg.: X. Mengual. Det.: X. Mengual. | EU241728 | EU241775 | EU241826 |
| *Allograpta* (*Rhinoprosopa*) *aenea* (Hull, 1937) | XP79 | COSTA RICA, P.N. Tapantí, Site 2. 11-I-2005. 1500 m. Leg.: G. Ståhls. Det.: X. Mengual. | EU241729 | EU241776 | EU241827 |
| *Allograpta* (*Rhinoprosopa*) *neonasuta* (Bigot, 1884) | XP91 | COLOMBIA, Dpto Valle del Cauca. Palmira, Corrg. La Buitrera. Nirvana. 14-II-2006. 1440-1530 m. Leg.: X. Mengual. Det.: X. Mengual. | EU241730 | EU241777 | EU241828 |
| *Allograpta* (*Rhinoprosopa*) sp. 5 | XP145 | COSTA RICA, Sitio de Flores. 13-VIII-2006. Det.: INBio staff. | EU241731 | EU241778 | EU241829 |
| *Allograpta* (*Rhinoprosopa*) *sycorax* (Hull, 1947) | XP147 | COSTA RICA, INBio code: 3439. Det.: INBio staff. | EU241732 | EU241779 | EU241830 |
| *Asarkina* (*Asarkina*) *ericetorum* (Fabricius, 1781) | S222 | KENYA, Kakamega forest, 5-XII-1995, 0°17.13'N 34°56.32'E. Leg.: Earthwatch Team 6. Det.: G. Ståhls. | EF127353 | EF127434 | EU241837 |
| *Asarkina* (*Asarkina*) *fulva* Hull, 1941 | XP100 | MADAGASCAR, Fianarantsoa Prov. Ranomafana N.P., Talatakely region. 22-XI-2004. Leg.: X. Mengual. Det.: X. Mengual. | EU241738 | EU241785 | EU241838 |
| *Asarkina* (*Asarkina*) sp. | XP99 | MADAGASCAR, Fianarantsoa Prov. Ranomafana N.P., Talatakely region. 27-XI-2004. Leg.: X. Mengual. Det.: X. Mengual. | EU241739 | EU241786 | EU241839 |
| *Asarkina* (*Asarkina*) *tenebricosa* Keiser, 1971 | XP50 | MADAGASCAR, Fianarantsoa Prov. Ranomafana N.P., Maharira Mountains. 28-XI-2004. Leg.: X. Mengual. Det.: X. Mengual. | | EU241787 | |
| *Episyrphus* (*Episyrphus*) *balteatus* (De Geer, 1776) | XP153 | SPAIN, Alicante. P.N. Marjal Pego-Oliva, Muntanyeta Verda. 19-V-2007. Leg.: X. Mengual. Det.: X. Mengual. | EU241740 | EU241788 | EU241840 |
| *Episyrphus* (*Episyrphus*) *meliscaevoides* Ghorpade, 1981 | XP52 | MADAGASCAR, Fianarantsoa Prov. Road from Valbio to Ranomafana city. 22-XI-2004. Leg.: X. Mengual. Det.: X. Mengual. | EF127319 | EF127398 | EU241841 |
| *Episyrphus* (*Episyrphus*) *viridaureus* (Wiedemann, 1824) | XP173 | EAST TIMOR, Maliana, road verge in town. S 8°58'51'' E 125°13'08''. 200 m. 11-XII-2005. Leg.: M.P. van Zuijen. Det.: M.P. van Zuijen. | EU241741 | EU241789 | EU241842 |
| *Exallandra cinctifacies* (Speiser, 1910) | XP148 | KENYA, Aberdares Nat. Park. 31-XII/14-I-2006. Malaise trap. Det.: F.C. Thompson. | EU241742 | EU241790 | EU241843 |
| *Meliscaeva auricollis* (Meigen, 1822) | S123 | GREECE, Lesbos island, IV-2001. Leg.: S. Rojo & C. Pérez. Det.: L. Mazanek. | EF127341 | EF127423 | EU241844 |
| *Meliscaeva cinctella* (Zetterstedt, 1843) | 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) | 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* (*Pipunculosyrphus*) *tiarella* (Hull, 1944) | 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 antiphates* (Walker, 1849) | 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 |
| *Ocyptamus funebris* Macquart, 1834 | XP85 | COLOMBIA, Dpto. Cauca, Corrg. El Tambo, 20 De Julio. 2900 m. 6/8-III-2006. Leg.: C. Prieto. Det.: X. Mengual. | EU241745 | EU241793 | EU241848 |
| *Salpingogaster* (*Eosalpingogaster*) *conopida* (Philippi, 1865) | 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*) *cornuta* Hull, 1944 | XP78 | COLOMBIA, Dpto. Cauca, Corrg. El Tambo, 20 De Julio. 2900 m. 6/8-III-2006. Leg.: C. Prieto. Det.: X. Mengual. | EU241746 | EU241794 | EU241851 |
| *Salpingogaster* (*Salpingogaster*) *CR-9* Thompson, in litt. | XP74 | COLOMBIA, Dpto. Cauca, Corrg. El Tambo, 20 De Julio. 2900 m. 6/8-III-2006. Leg.: C. Prieto. Det.: X. Mengual. | EU241747 | EU241795 | EU241852 |
| *Salpingogaster* (*Salpingogaster*) *nigra* Schiner, 1868 | 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 | 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*) *contigua* Macquart, 1847 | XP97 | USA, MO: Barry Co. Cassville, 7 mi. S. Rouring River SP. 12-IV-2004. Leg.: J. & W. van Steenis. Det.: W. van Steenis. | EU241750 | EU241798 | EU241855 |
| *Sphaerophoria* (*Sphaerophoria*) *loewii* Zetterstedt, 1843 | S273 | SWEDEN, Upplands-Bro, 15-VI-2002. Leg.: H. Bartsch. Det.: G. Ståhls. | EF127318 | EF127396 | EU241856 |
| *Sphaerophoria* (*Sphaerophoria*) *macrogaster* (Thomson, 1869) | XP44 | AUSTRALIA, Jimbour Qld. 11-IX-2003. Leg. L.W. Smith. Host: Capitophorus elaegni on Silybum marianum. Det.: L.W. Smith. | | EU241799 | EU241857 |
| *Sphaerophoria* (*Sphaerophoria*) *philanthus* (Meigen, 1822) | XP41 | CANADA, AB. Jasper NP. Valley o/t Five Lakes. 117°98'E 52°48'N. 27-VIII-2004. Leg. W. van Steenis. Det.: W. van Steenis. | EU241751 | | EU241858 |
| *Sphaerophoria* (*Sphaerophoria*) *rueppellii* (Wiedemann, 1830) | S12 | SPAIN, Alicante, 1999. Det.: S. Rojo. | EF127328 | EF127409 | EU241859 |
| *Sphaerophoria* (*Sphaerophoria*) *scripta* (Linnaeus, 1758) | XP142 | SPAIN, Alicante, Aspe. Partida Tolomó.07-II-2006. Leg.: P. Hurtado. Det.: X. Mengual. | EU241752 | EU241800 | EU241860 |
| *Toxomerus flaviplurus* (Hall, 1927) | 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 mutuus* (Say, 1829) | 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 politus* (Say, 1823) | XP82 | COSTA RICA, P.N. Tapantí. 12-I-2005. 1600 m. Leg.: S. Rojo. Det.: F.C. Thompson. | EU241755 | EU241803 | EU241863 |

noted as e.g. *CR-5*, *CR-7*. There is a world revision of the genus *Allograpta* still unpublished (Mengual et al., unpublished) where all these species have been studied.

### 2.2. Laboratory procedures

DNA was extracted from legs or other parts of single individuals of either dry, pinned or ethanol preserved specimens using the NucleoSpin Tissue DNA Extraction kit (Machery-Nagel, Düren, Germany) following manufacturer's protocols and resuspended in 50 μl ultra-pure water. Remnants of specimens were preserved as DNA voucher specimens for the purpose of morphological studies and deposited at Finnish Museum of Natural History (MZH) and labeled as listed in Table 2.

PCRs (25 μl) included 3–4 μl DNA extract, 1 μl of each primer, (at 10 pmol/μl), 0.25 μl of Taq DNA polymerase (5 U/μl), 2 μl 2.5 mM $MgCl_2$, 2.5 μl 10X Buffer II (Applied Biosystems, Foster City, CA, USA) and 4 μl 200 mM dNTP (GeneAmp) and ultra-pure water. The PCRs involved an initial denaturation step (95 °C for 2 min) following by 29 cycles of 30 s denaturing at 94 °C, 30 s annealing at 49 °C, 2 min extension at 72 °C, followed by a final extension of 8 min at 72 °C. The double-stranded PCR products were visualized by agarose gel electrophoresis (1.5% agarose). The universally conserved primers used for amplifying and sequencing the COI, 18S and 28S fragments are listed in Table 3. Generally, the COI fragment was amplified in 2 fragments using the forward primer LCO1-1490 and the reverse primer HCO1-2198 for the first part, and the forward primer C1-S-1718 (alias Beet) and the reverse primer TL2-N-3014 (alias Pat). The homologous COI sequence fragment could also be obtained by using primer combinations C1-S-1718 + C1-N-2735 (alias Inger) and C1-J-2183 (alias Jerry) + TL2-N-3014 using the above PCR and sequencing conditions (see Table 2). The D2-3 region of the nuclear 28S rRNA gene was amplified with the primers and PCR profiles described in Belshaw and Quicke (1997) and Campbell et al. (1993) (see also Laurenne et al., 2006). The nuclear 18S rRNA gene was partially amplified using the primers 18s 2F and 18s b2.9, both designed from insect sequences by The Crandall Lab (see also Whiting et al., 1997; Whiting, 2002), following the same PCR profiles.

The double-stranded PCR products were visualized by agarose gel electrophoresis (1.5% agarose), and purified using the GFX PCR Purification Kit (Amersham Biotech, Little Chalfont, UK). Amplified PCR was checked for size and products (bands) running 4 μl on a 1.5% agarose gel and then sequenced (with the PCR primers) in both directions using the Big Dye Terminator Cycle Sequencing Kit vs. 1.1 (Applied Biosystems) at one-fourth of the recommended volumes on an ABI PRISM 377 (Applied Biosystems) sequencer.

Chromatograms were read and edited for base-calling errors and assembled using Sequence Navigator™ (version 1.01, Applied Biosystems). All new sequences were submitted to GenBank, see Table 2 for accession numbers.

### 2.3. Phylogenetic analyses

#### 2.3.1. Parsimony

Parsimony analyses were carried out using two different approaches: optimization alignment and static alignment. The first approach optimizes sequence variation directly during cladogram searching, and eliminates the necessity to align sequences prior to analysis. Static alignment refers to methods where multiple sequence alignments are performed to create primary homology statements followed by a tree search using standard techniques.

*2.3.1.1. Direct optimization.* Datasets were analyzed with POY version 3.0.11 (Wheeler et al., 2003) using the direct optimization method with parsimony as the optimality criterion (Wheeler, 1996). Direct optimization searches for the shortest tree using unaligned sequences as input (which may be of unequal length) (Wheeler, 1996; Schulmeister et al., 2002; Wheeler et al., 2006a). The optimization algorithm incorporates indels (gaps) as events in the procedure in addition to base transformations. This allows searching for the shortest tree, the one that best explains the observed data, without the intermediate step of producing a multiple alignment (Aagesen, 2005).

The full dataset used for POY included five data partitions, three for the mitochondrial COI gene, one for the nuclear D2-3 28S rRNA gene region and one for the nuclear 18S rRNA. COI sequences were split into three arbitrary fragments identified from conservative primer defined areas, to speed up computation times. Sequences from ribosomal genes were not divided in fragments and were not aligned prior to analyses.

The parsimony analyses were run using a parallelized version of the program POY version 3.0.11 on a 21-node Beowulf cluster with 2.4 GHz processors employing Scyld Unix and parallel virtual machine (PVM) software at the Finnish Museum of Natural History, Helsinki, Finland. The following string of commands was used: -norandomizeoutgroup -noleading -parallel -replicates 100 -buildsperreplicate 3 -gap [1,2] -maxtrees 50 -holdmaxtrees 100 -seed -1 -slop 1 -checkslop 10 -treefuse -fuselimit 25 -drifttbr -numdrifttbr 5 -impliedalignment -indices. The analyses were performed using two different parameter sets of indel-transversion-transition ratios: equal weights [gap cost = 1, change cost = 1] and default values [gap cost = 2, change cost = 1]. Our purpose is not to explore the parameter space and to choose an optimal tree with the optimal cost value using the incongruence length difference, ILD (Mickevich and Farris, 1981; Farris et al., 1995) or its modifications as suggested by Wheeler (1995) (applied in Wheeler and Hayashi, 1998; Aagesen, 2005; Laamanen et al., 2005; Kutty et al., 2007; Schulze et al., 2007), but to explore if the clades obtained under equal costs are recovered under non equal parameter costs. The most dramatic change in the indel-transversion-transition ratio is probably found between equal weights and differential weights, but once gap and tv/ts costs are different, there is an infinite space where the unequal alignment parameter costs could be

**Table 3**
Primers used for amplifying and sequencing the COI, 28S and 18S fragments

| Gen fragment | Primer name | Sequence | Source |
|---|---|---|---|
| COI | C1-J-2183 (Jerry) | 5′-CAACATTTATTTTGATTTTTTGG-3′ | Simon et al. (1994) |
| | C1-S-1718 (Beet) | 5′-GGAGGATTTGGAATTGATTAGTTCC-3′ | Simon et al. (1994) |
| | TL2-N-3014 (Pat) | 5′-TCCAATGCACTAATCTGCCATATTA-3′ | Simon et al. (1994) |
| | C1-N-2735 (Inger) | 5′-AAAATGTTGAGGGAAAAAATGTTA-3′ | Lunt et al. (1996) |
| | LCO1-1490 | 5′-GGTCAACAAATCATAAAGATATTG-3′ | Folmer et al. (1994) |
| | HCO1-2198 | 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ | Folmer et al. (1994) |
| 28S | 28Sforward (F2) | 5′-AGAGAGAGAGTTCAAGAGTACGTG-3′ | Belshaw et al. 2001 |
| | 28Sreverse (3DR) | 5′-TAGTTCACCATCTTTCGGGTC-3′ | Belshaw et al. 2001 |
| 18S | 2F | 5′-AGGGTTCGATTCCGGAGAGGGAGC-3′ | The Crandall lab |
| | b2.9 | 5′-TATCTGATCGCCTTCGAACCTCT-3′ | The Crandall lab |

explored. Furthermore, the character and topological congruence indexes used to decide which parameter cost is optimal have proven problematic (e.g. Aagesen et al., 2005; Ramirez, 2006; Wheeler et al., 2006b) making this decision more difficult, even more when considering the philosophical aspects of the procedure (see Frost et al. (2001) and Grant and Kluge (2003, 2005) for a discussion). Bremer values (Bremer, 1988, 1994) were calculated for the most parsimonious trees using POY. The implied alignment from the unequal parameter cost search was used to calculate bootstrap support values (1000 replicates, 10 random additions per replication) performed in NONA (Goloboff, 1999a,b) spawn from Winclada (Nixon, 2002).

*2.3.1.2. Static alignments.* In order to evaluate the hypotheses under direct optimization vs. hypotheses derived from static alignments, we performed a more traditional two-step phylogenetic analysis of the data. Sequences of ribosomal genes 28S and 18S were aligned using the ClustalX (Thompson et al., 1997) with the defaults values for alignment parameters [gap opening cost 15, gap extension cost 6.66], with no prealignment for the protein-coding gene COI. The alignments were not adjusted by eye *a posteriori* to avoid the lack of objectivity and repeatability (Giribet and Wheeler, 1999).

The combined dataset of the three genes was analyzed in TNT 1.1 (Goloboff et al., 2007) performing a heuristic search of 300 replicates using TBR, followed by ratchet (Nixon, 1999), tree-drifting and tree-fusing (Goloboff, 1999a,b). Branch support values (Bremer support) were estimated for the most parsimonious trees. All trees were drawn with the aid of Winclada (Nixon, 2002).

*2.3.2. Bayesian inference and maximum likelihood*

Although there is no justification for pluralism in phylogenetic systematics (Giribet et al., 2002), data sets may be extremely sensitive to parameters or models. For this reason, we were interested to use maximum likelihood analyses and Bayesian inference for our data. For the entire concatenated data set—using the same ClustalX alignment—we determined the best choice of model using ModelTest 3.7 (Posada and Crandall, 1998) under the Akaike Information Criterion (AIC), as recommended by Posada and Buckley (2004). The model chosen was GTR + I + G, and this model was also chosen under the Bayesian Information Criterion (BIC) for the same data. We analyzed the data under the recommended model using Garli v0.951 (Zwickl, 2006) as shortcut to obtain an initial topology (5 times in total obtaining always the same result), doing one final tree search in PAUP* version 4.0b10 (Swofford, 2003) using maximum likelihood as an optimality criterion. Finally, we analyzed our datasets using MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001) under the recommended model. Four chains were employed for each run, and 1,000,000 generations were sufficient to bring the convergence to a value <0.01.

## 3. Results

### 3.1. Data

Sixty-four ingroup taxa and one outgroup taxon were included in the analysis (Table 2). The mitochondrial COI dataset comprised 1382 nucleotide characters, and this gene could not be amplified for two taxa (*Asarkina tenebricosa* and *Sphaerophoria macrogaster*), and 21 taxa are missing a fragment of 253 nucleotides of the 5′ prime region. 444 nucleotide sites were parsimony informative. The mean AT-content of the COI sequences was 70.40%. The uncorrected pairwise sequence divergences for the COI gene between the species of *Allograpta* ranged from 0.0%, between *A.* (*Fazia*) *CR-5* and *A.* (*Fazia*) *centropogonis*, to 17.06%, between *A.* (*Allograpta*) sp. 1 and *A.* (*Fazia*) *fascifrons* [XP25]. Intra-subgeneric divergences ranged from 0.16% to 6.11% in *Allograpta* (*Rhinoprosopa*); between 0.0% and 12.7% in *Allograpta* (*Fazia*); and from 0.0% to 9.56% in *Allograpta* (*Allograpta*).

The ribosomal D2–3 region of 28S rRNA gene was obtained for 64 included taxa and comprised 582–602 nucleotide sites. The sequencing of 18S rRNA produced a sequence fragment of 687 nt, from which we removed approx. 330 nt fragment that was identical among all included terminals, resulting in a 350–355 nt fragment used for analyses. This gene region could not be amplified for five taxa (see Table 2).

### 3.2. Phylogenetic analyses

*3.2.1. Direct optimization*

The POY analysis using equal weighting values (1:1) resulted in one most parsimonious tree (MPT) with 3403 steps (CI = 0.10, RI = 0.81), shown in Fig. 1. During the course of the study, the parsimony analysis was run multiple times changing several parameters, like increasing values for -replicates -buildsperreplicate -slop and -checkslop commands (see Wheeler et al., 2006a for explanation of the commands). In all the analyses using equal weights the same most parsimonious tree was found, independent of alterations of the parameter values.

The MPT recovered the genus *Allograpta* comprising two clades, the first or *upper* clade (only indicates topological position in the tree; see Fig. 1) with *A.* (*Antillus*) *ascita* as sister group of two groups, the subgenera *Rhinoprosopa* + CR, and part of the members of *Fazia*. The second or *lower* clade showed *A.* (*Allograpta*) *fuscotibialis* and *A.* (*Allograpta*) *obliqua* (the type species of *Allograpta*) in a sister group position with respect to the remaining taxa which were resolved into three clades: (1) species of subgenus *Allograpta* s. str.; (2) *Sphaerophoria* + *Exallandra*; and (3) remaining members of the subgenus *Fazia*. All clades were recovered with moderate to high Bremer support values.

Under differential weighting (2:1), three most parsimonious trees (with 3524 steps of length; CI = 0.11, RI = 0.82) were recovered in all the analyses, independent of alterations of the parameter values. The topology of the strict consensus tree (Fig. 2) is basically the same as the MPT under equal weights. The differences were in the position of the genus *Salpingogaster* s. str., that in the equal weighting tree was placed as sister to (((*Asarkina* + (*Sphaerophoria* + *Exallandra*)) + *Allograpta* sensu lato), while in the differential weighting tree was placed as sister to *Allograpta* sensu lato (including *Sphaerophoria* and *Exallandra*). The other difference was found in the *lower* clade of *Allograpta*: *A. fuscotibialis* + *A. obliqua* were resolved as sister to *Sphaerophoria* + *Exallandra*, while the equal weighting recovered the taxa as sister to all taxa of the *Allograpta* "lower" clade.

*3.2.2. Static alignments*

Parsimony analyses performed with TNT 1.1 resulted in six equally parsimonious trees, length 3478 steps. The topology of the strict consensus tree (3485 steps) is virtually the same as the MPT obtained in the non equal weighting analysis (Fig. 2) using POY (the same strict consensus tree was also recovered running a parsimony analysis in PAUP*). The recovered clades comprise the same members but there is one change: the sister relationship between *A.* (*Antillus*) *ascita* and the subgenera CR and *Rhinoprosopa* obtained with TNT was not recovered in other analyses. Branch support values were high for the same nodes as in the 1:1 and 2:1 direct optimization cladograms.

*3.2.3. Maximum likelihood and Bayesian inference*

*Sphaerophoria* and *Exallandra* were also recovered within *Allograpta* using maximum likelihood implemented in PAUP* under GTR + I + G model. The best maximum likelihood tree (tree

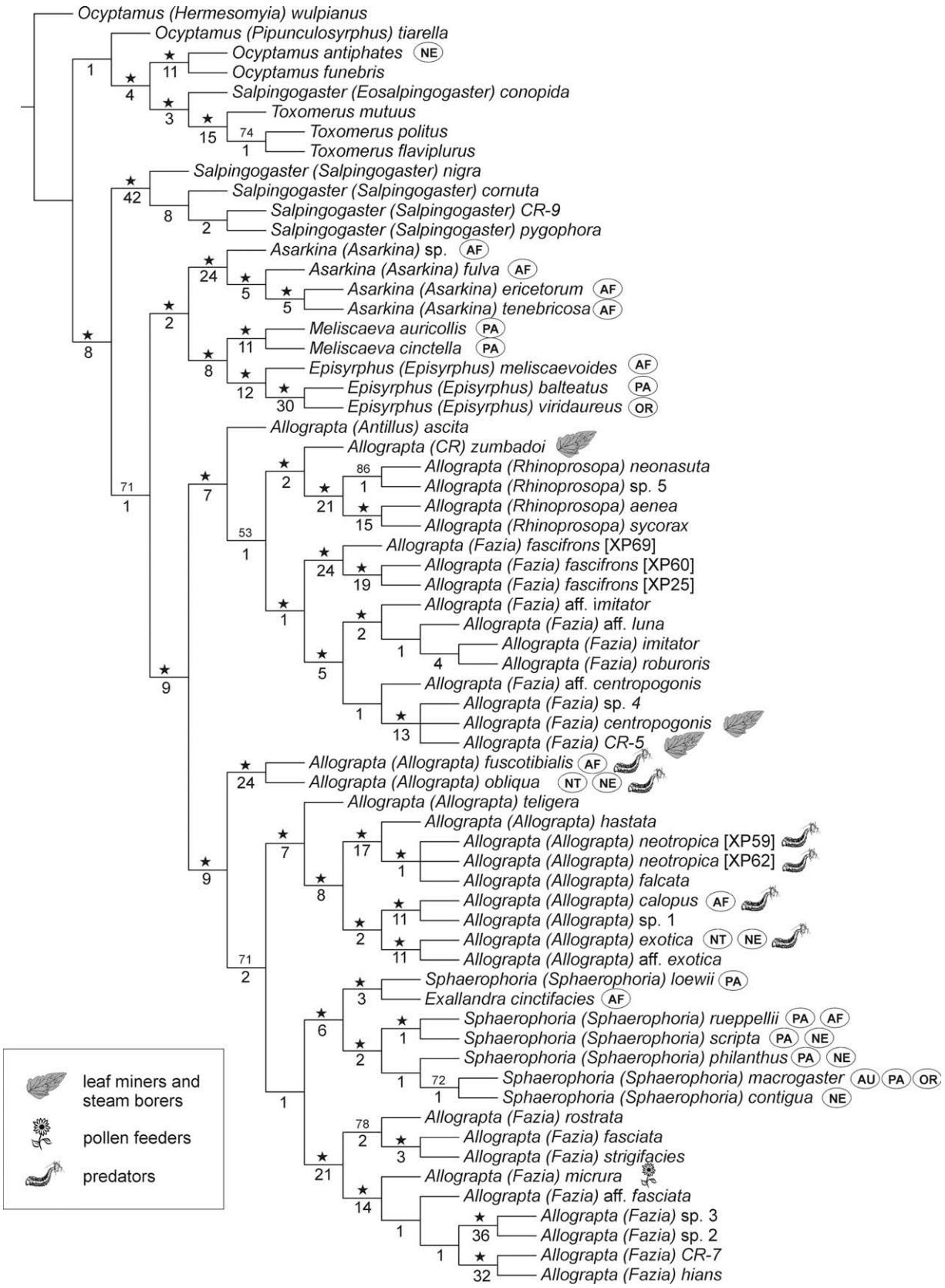


**Fig. 1.** Most parsimonious tree inferred from sequences of COI, 28S and 18S genes using direct optimization in POY (length = 3403 steps; CI = 0.1, RI = 0.81). Gap cost = 1, indel cost = 1. Bremer support values are indicated below branches. Stars indicate nodes supported with greater than 95% posterior probability in the Bayesian inference. Non Neotropical species are indicated by their biogeographical region (AF, Afrotropical; AU, Australasian; NE, Nearctic; NT, Neotropical; OR, Oriental; PA, Palaearctic). If the species is present in more than one region, it is also indicated. Species without indicated region are Neotropical taxa. Known larval feeding modes of *Allograpta* species are indicated.

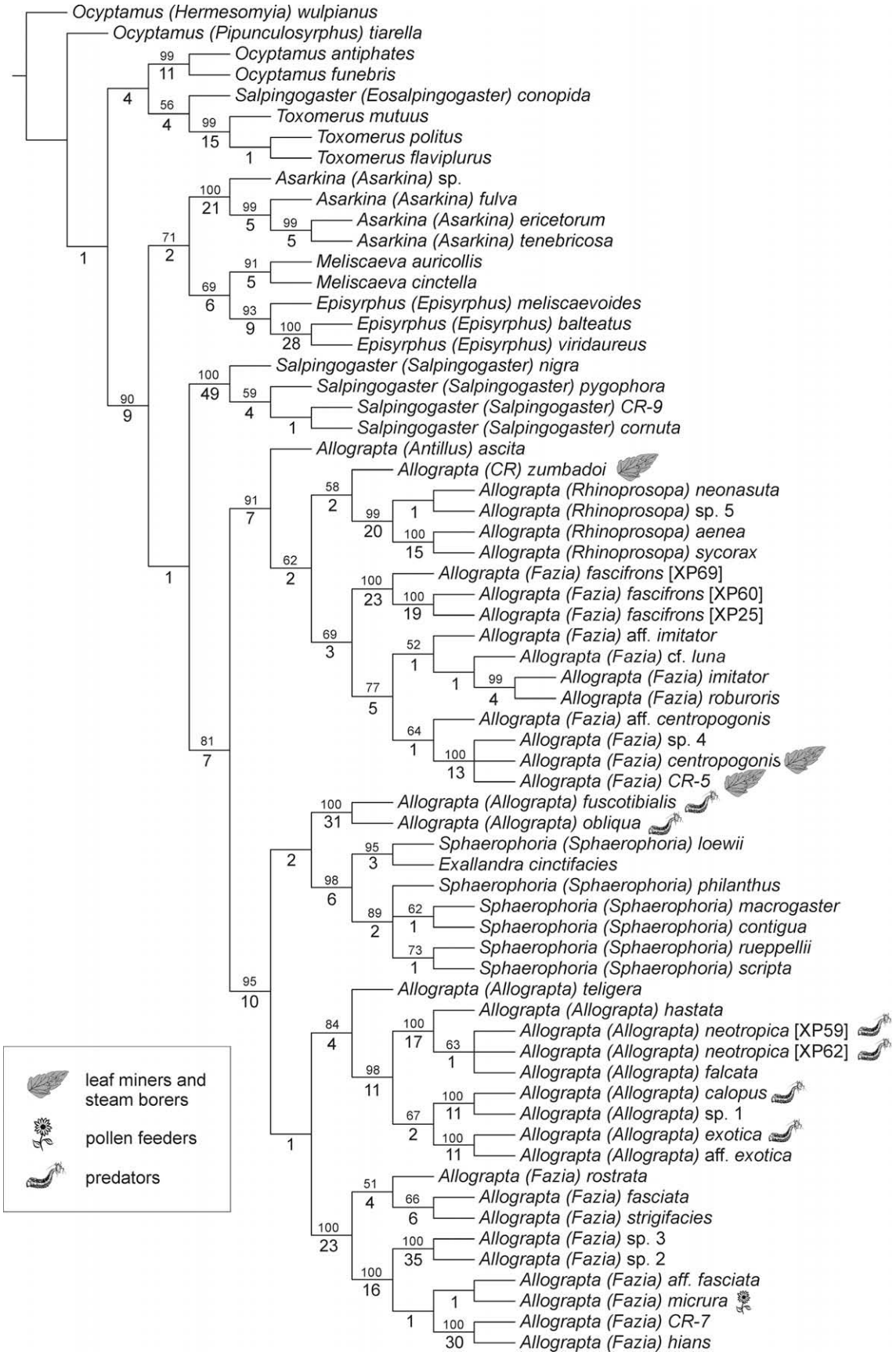


**Fig. 2.** Strict consensus tree of three most parsimonious trees based on COI, 28S and 18S genes using direct optimization in POY. Gap cost = 2, indel cost = 1. Bremer support values are indicated below branches; bootstrap support values (>50%) are indicated above branches. Known larval feeding modes of *Allograpta* species are indicated.

score = −19060.092) is identical to the cladogram using equal weights in direct optimization with parsimony as optimality criterion (see Fig. 1).

For the Bayesian inference, the first 200,000 generations of the Markov chain Monte Carlo (MCMC) were defined as "burn-in", using the remaining generations for calculating the posterior probabilities of the clades. The topology of the tree using MrBayes is almost identical compared with the MPT using POY and equal weights (see Fig. 1). The main difference is the placement of *Sphaerophoria* + *Exallandra* clade, sister to *Allograpta* s. str. + part of *Fazia*, while the equal parameter cost analysis recovered them as sister to part of *Fazia*. High values of posterior probability are found for most of the clades that were also recovered in the parsimony analyses (see Fig. 1, Bayesian PP values >95% indicated with *).

*3.2.4. Phylogenetic trees*

A summary of the clades recovered in all the analyses is presented below. *Ocyptamus* (*Pipunculosyrphus*) *tiarella* was placed as sister group of all the ingroup taxa. (*Ocyptamus* + (*Salpingogaster* (*Eosalpingogaster*) *conopida*) + *Toxomerus*)), (*Asarkina* + (*Episyrphus* + *Meliscaeva*)), and the species of *Salpingogaster* s. str. were groupings consistently found.

Based on our results, the tribe Syrphini was not recovered as monophyletic due to the presence of the tribe Toxomerini in close relationship with the genus *Ocyptamus* and the subgenus *Eosalpingogaster*. This result agrees with Mengual et al. (2008) where a more global view of the subfamily is given and the problems of the current tribal classification of Syrphinae are discussed.

*Salpingogaster* s. str. constitutes a monophyletic group in all the trees obtained from different approaches and parameter schemes, with the exclusion of subgenus *Eosalpingogaster* which is recovered embedded in the (*Ocyptamus* + *Toxomerus*) clade. Mengual et al. (2008) only included a single species of *Salpingogaster*, *S.* (*Eosalpingogaster*) *conopida*, in their analysis which was also recovered in relation with *Ocyptamus*. Thus, monophyly of *Salpingogaster* is not supported in this analysis and the obvious need for a broader study about the monophyly and placement of this genus will be addressed in future publications.

The genus *Allograpta* was consistently found to be paraphyletic with respect to *Sphaerophoria* + *Exallandra*, and the clade received high Bremer support in every parsimony analysis. In the study of Mengual et al. (2008), *Sphaerophoria* species were also placed within the genus *Allograpta*, but *Exallandra* was not studied. *Allograpta* species were divided in two big clades. The subgenera *Antillus*, CR, *Rhinoprosopa* and part of *Fazia* species in one group, and the typical *Allograpta* subgenus with the other *Fazia* species plus genera *Sphaerophoria* and *Exallandra* in the other clade. The differences in the phylogenetic placements within *Allograpta* sensu lato are reported below.

The trees obtained under different approaches all show ((*Antillus* + (*Rhinoprosopa* + CR) + (part of *Fazia*)). The monophyly of subgenera *Fazia* and *Allograpta* is not supported. *Fazia* is divided in two parts indicating that the diagnostic characters of forwardly produced face and the pilose metasternum are not unique to one subgenus, and *Allograpta* s. str. was also recovered as two lineages. In Mengual et al. (2008) the number of species was reduced and only the non-monophyly of the subgenus *Allograpta* was observed. The species of *Fazia* included in Mengual et al. (2008) were also placed together in our analyses.

In all analyses *A.* (*Fazia*) *centropogonis* is resolved in politomy with *A.* (*Fazia*) *CR-5* and *A.* (*Fazia*) sp. 4. External morphology of these species is not informative for distinguishing them, but characters of the male genitalia can differentiate these closely related species.

*Allograpta* (*Fazia*) *fascifrons* (specimens XP25, XP69 and XP60) constitutes a species group that shares some morphological characters of the head with the monotypic sg. *Claraplumula* and thus specimens key out to this subgenus (unpublished identification key by F.C. Thompson), but based on comparison of multiple other characters they are not *A. (Claraplumula) latifacies*. Morphological and molecular analysis of *A. latifacies* is needed to shed more light on the phylogenetic affinities and rank of this species group.

In the subgenus *Allograpta*, *A. fuscotibialis* and *A. obliqua* (type species of the genus) were always recovered separately of the rest of the subgenus. The placement of *A. fuscotibialis* and *A. obliqua* as a sister group of subgenera *Allograpta* and *Fazia* plus *Sphaerophoria* and *Exallandra* is unexpected, but well-supported in all analyses under different parameters and approaches. In parsimony analyses, using static alignment and direct optimization with unequal alignment parameter costs, these species were placed differently as sister group of *Sphaerophoria* + *Exallandra*.

*Allograpta* (*Allograpta*) *falcata* was always recovered in a polytomy with two specimens of *A.* (*Allograpta*) *neotropica*. No differences were found in COI gene nor in the 18S and 28S genes explored between both species, and no morphological characters can distinguish them except the darker colour of *A. falcata* (Thompson, personal communication). Our results agree with his perception that *A. falcata* is possibly only a darker form of *A. neotropica*.

## 4. Discussion

### *4.1. Taxonomy of Allograpta and Sphaerophoria*

Results of the present analyses show without doubts that genera *Sphaerophoria*, *Exallandra* and *Allograpta* share a common evolutionary history. The distributions of the genera *Sphaerophoria* and *Allograpta* are, in some way, complementary. *Sphaerophoria* is mainly found in the Holarctic, Oriental, and Australian regions (+ 2 species cited from Ethiopian region; Vockeroth, 1973b), while *Allograpta* is almost worldwide, absent from northern Nearctic and from Palaearctic except its south-eastern portion (Vockeroth, 1969). Furthermore, where the diversity of one genus is high (Holarctic for *Sphaerophoria*, Neotropic and Afrotropic for *Allograpta*) the presence of the other is null or very reduced (Neotropic/Afrotropic for *Sphaerophoria* and Western Palaearctic for *Allograpta*). But, in the areas where both taxa are present, the Australasian or Oriental regions, the elevated diversity of species of *Allograpta* [21 species in Australasian region, nine species in the Orient, 12 in Africa] contrasts with the low or medium number of species of *Sphaerophoria* [one species in Australia, 12 in the Orient, four in continental Africa] (Vockeroth, 1969, 1973b; Thompson, 2006). Only the typic subgenus of *Allograpta* is distributed outside the Neotropical region, but the greatest diversity is found in the Neotropics with 56 described species (Thompson, 2006). These data could suggest that the two genera are quite distinct, as Vockeroth (1969) commented, or the existence of a genus with worldwide distribution with species diversification in almost all biogeographical regions.

The present study showed the necessity to also include taxa from Australia and New Zealand and morphological characters for an improved estimate of the subdivision of *Allograpta* sensu lato, and for resolving the taxonomic status of new species. Our significant results are unequivocal about the close relationships of *Sphaerophoria*, *Exallandra* and *Allograpta*, but the decision to consider them as members of a single genus (*Sphaerophoria* in this case) or split them in several genera must wait for further analyses, as also a subgeneric classification based on a comprehensive taxon sampling and a combined study of morphological and molecular characters.

### *4.2. Taxonomy of Sphaerophoria and Exallandra*

Our results indicate that *Sphaerophoria* is a paraphyletic genus in terms of *Exallandra*. Optimization and static alignment using

parsimony, maximum likelihood and Bayesian inference always recovered *Sphaerophoria* as a clade with *Exallandra cinctifacies* nested within it, consistently grouped with *Sphaerophoria loewii*. Vockeroth (1969) indicated that the terminalia of *S. novaeangliae* and *S. loewii* do differ rather markedly from those of the other species. Our results present *S. loewii* as sister group to *Exallandra cinctifacies*.

*Exallandra cinctifacies* was described by Vockeroth (1969) for a species from Ethiopia (now also known from Kenya, Liberia, Congo and South Africa), which resembled *Sphaerophoria* but could be separated from this by the much smaller male terminalia and by the *Syrphus*-type abdominal colour pattern. Vockeroth (1969) noted that *Exallandra* is extremely similar in external structural characters to some *Melangyna* species, but again used the size of the male terminalia and colour pattern, and the disjunct distribution to distinguish them. As previously noted, the differences in size of the male genitalia were also used by Vockeroth (1969) to separate *Allograpta* from *Sphaerophoria*. Based on present results, the mere size of the male genitalia is not a phylogenetically informative trait, as *Exallandra* and *Allograpta* s. str. have small sized genitalia and many *Sphaerophoria* have large. Vockeroth (1969) indicated the subscutellar hair fringe as a useful character to separate these genera: *Allograpta* with a complete hair fringe, reduced in *Exallandra* and absent in *Sphaerophoria*. But the species *Allograpta* (*Fazia*) *centropogonis* has incomplete subscutellar hair fringe not facilitating the separation of these taxa based on this character. In congruence with our findings, the synonymy of genus *Exallandra* with *Sphaerophoria* seems obvious, but more morphological characters and a higher number of *Sphaerophoria* species should be analyzed for a well-founded conclusion.

### 4.3. Genus Allograpta

All the analyses using different approaches always recovered a monophyletic *Allograpta* clade, where *Sphaerophoria* and *Exallandra* were deeply embedded.

Our molecular results were not congruent with previous subgeneric classification, based on morphological characters (Vockeroth, 1969, 1973a), because subgenera *Fazia* and *Allograpta* s. str. were not recovered as monophyletic groups. Mengual et al. (unpublished) review the current classification of the genus *Allograpta* including all the known species from the different biogeographical regions, and providing a new name for the group of stem-boring species (here called subgenus CR). The six subgenera system used in our study is based on their work and knowledge, but our results using molecular data do not agree with their reviewed classification neither.

Vockeroth (1969) established a new genus for *Antillus ascitus* (from Haiti and Dominican Republic) which was synonymized with *Allograpta* by him years later (Vockeroth, 1973a). *Rhinoprosopa* was given by Hull (1942) as a new name for the species *Oligorhina aenea* (Hull, 1937) and kept in the tribe Bacchini due to its petiolate abdomen until Vockeroth (1973a) decided to synonymize it under *Allograpta* due to the synapomorphies it shared (see Carver and Thompson, 2003). Subgenus CR is a new subgenus designated for two very distinct species, which are plant-feeders, only found in Costa Rica (Mengual et al., unpublished). These subgenera of *Allograpta* are readily distinguished from *Sphaerophoria*, *Exallandra* and *Allograpta* s. str. by their facial shape (anteriorly extended) and autapomorphic characters (apical wing maculae, petiolate abdomen, well developed plumula) (Mengual et al., unpublished).

#### 4.3.1. Larval feeding modes

A hypothesis about the time and the manner of colonization of South America by Syrphini is given by Vockeroth (1969). Aphids (Aphididae), a major prey group of syrphines in the Holarctic region, were mainly introduced in Neotropical region by human activities and displacements during the last 500 years (see Dixon, 1998; Blackman and Eastop, 2000), and by natural invasion during recent geological periods (Ortego et al., 2006; and references therein), but in the Neotropical region only few species of aphids exist (Dixon, 1998; Nieto Nafría et al., 2002).

The very low abundance of aphids in Neotropics has resulted in exploitation of other food sources. The Neotropical region offers a multitude of different ecological niches that could spark a rapid and big diversification of larval feeding modes. In Neotropics non common syrphid prey taxa like larvae of craneflies (Tipulidae), mosquitoes (Culicidae), and aquatic beetles (Coleoptera, Helodidae) have been recorded (Rotheray et al., 2000), a fact that shows the ecological plasticity of the family Syrphidae. The availability of new food sources allowed *Allograpta* spp. to change predation for phytophagy with apparently some changes in larval morphology, such as the acquisition of a toothed labium and labrum in *Allograpta centropogonis*. Most of the features of known *Allograpta* larvae are shared with other syrphine genera which could demonstrate their generally unspecialized morphology. Greater morphological diversity has been observed in other syrphine lineages such as the genus *Ocyptamus*, which comprises multiple larval feeding modes e.g. feeding on aphids and coccids on the leaf surface of plants, and subaquatic predation (Rotheray et al., 2000). Hence, for *Allograpta*, there is no reason to suppose that this genus could not also have developed higher levels of specialized morphology and radiated into a variety of ways of life.

The biology of some species of the subgenus *Allograpta* s. str. is known, e.g. *A. fuscotibialis*, *A. obliqua*, *A. exotica*, *A. calopus* and *A. neotropica* feed mainly on aphids (families Adelgidae, Aphididae) but also on whiteflies (Aleyrodidae), mealybugs (Pseudococcidae), larvae of Lepidoptera, spider mites (Acari) and psyllids (Psyllidae) (Rojo et al., 2003). The most diverse prey range is found for *A. calopus* (South Africa), *A. javana* (Oriental species; India to Japan) and *A. obliqua* (mainly Nearctic species but also introduced in Hawaii) (Thompson, 2006). Based on the literature, it would seem reasonably to treat *Allograpta* as a predatory taxon, except that four phytophagous species are known (see below), but this reasoning could be biased by the current knowledge of the biology of larvae.

The known plant-feeder species of subgenera *Fazia* and CR in the *upper* group are placed together in this clade with high support values, a fact that could reveal a monophyletic lineage with the same larval feeding biology. *Allograpta centropogonis* and the undescribed species *Allograpta CR-5*, shown to be plant-feeder by Thompson (personal communication), are known to be leaf miners. Larvae of *Allograpta centropogonis* has several unique morphological features but not unexpected, considering that a shift from a predaceous ancestor has probably occurred (Nishida et al., 2002). For example, the head skeleton is of the usual syrphine type except that in *A. centropogonis*, there are rows of dorsal and ventral hooks at the apex and the mandibles are apparently reduced or lost (Nishida et al., 2002). We hypothesize that this subgroup of *Fazia* could constitute a monophyletic clade of plant-feeders, which future studies will support or refute.

In the *lower Allograpta* clade, the members of subgenus *Fazia* constitute a monophyletic group. Of the included species the larval feeding mode is only known from *A.* (*Fazia*) *micrura*, reported as a pollen-feeder by Weng and Rotheray (unpublished). Although this is phytophagy, the use of the vegetal matter is completely different of the strategy of species of the subgenus CR or *A. centropogonis*, but this switch in larval feeding habit from predation to pollen feeding is not exclusive for *Allograpta*, as *Toxomerus politus*, a species in a genus also comprising aphid predators, was also reported as pollen-feeder too (see Rojo et al., 2003 and references therein).

The evolutionary scenario presented in this analysis indicates that phytophagy has evolved at least two times in the history of

the group. The phytophagous larval feeding mode is different in both cases, stem-boring and leaf-mining versus pollen feeding. Finally, the controversial observation of *Allograpta obliqua* larvae being able to sustain themselves on plant food (Davidson, 1922) prompts us to hypothesize a more complex pattern of evolution of phytophagy in *Allograpta* that would include obligatory and facultative larval feeding biologies. Future studies on the morphology of adults and larvae, and more field work for finding larvae of the *Allograpta* species will improve our knowledge on this interesting syrphid genus, and will confirm or refute the hypothesis of multiple origins for phytophagy.

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