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First report on effective pollination of *Masdevallia floribunda*, *M. tuerckheimii* and their hybrid (*Orchidaceae-Pleurothallidinae*) by *Zygothrica* fruit flies (*Diptera-Drosophilidae*) in Guatemala

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with 3 figures

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Summary

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The first report on pollination of two Neotropical orchid species, *Masdevallia floribunda* Lindl., *M. tuerckheimii* Ames and their hybrid by fruit flies is presented. Two presumably undescribed species of the genus *Zygothrica*, tentatively named as *Zygothrica* spec. 1 and spec. 2, were observed as pollinators. The distinct hypercephaly in the examined males, the transparent wing membrane lacking infuscations or markings in both sexes indicate that *Zygothrica* spec. 1 is a member of the *caudata* subgroup of the *dispar* species group. The exact affinities of *Zygothrica* spec. 2 are uncertain, but this species resembles *Z. mesopoeyi* Burla. Our observations evidenced that the *Masdevallia* flowers attract both males and females of *Zygothrica*, and that both can carry the pollinia. The Estación Experimental de Orquídeas de la Familia Archila, a seminatural plantation located in a cloud forest of Guatemala, is a place of intense pollination activity by *Zygothrica* adults on the flowers of the two *Masdevallia* species, which also leads to effective and frequent hybridization.

1. Introduction

Orchids are well known as one of the most advanced groups of flowering plants in the context of adaptation to different forms of zoogamy (especially entomogamy). The pollination by flies (myophily), with all its variations, is the second most common pollination pathway in the *Orchidaceae*, with pollination by species of about 20 families of *Diptera* being known (Christensen 1994). According to different estimates (e.g. van der Pijl & Dodson 1966, Christensen 1994), 15 to 25 % of orchid species are pollinated primarily by flies. One of the two largest myophilous groups among the orchids is the Neotropical subtribe *Pleurothallidinae* Lindl. (van der Pijl & Dodson 1966).

Many studies have proven the high specificity of relationships in pollination systems in *Orchidaceae*, but these mostly concern species pollinated by groups of *Hymenoptera* (for instance, euglossine bees; e.g. Dressler 1968, Williams & Dodson 1972, Ackerman 1983), the pseudocopulatory mechanism in *Ophrys* L. (Borg-Karlson 1987, Paulus & Gack 1990), and pollination by ants in *Zosterophyllanthos* Szlach. & Marg. (Archila & Bertolini 2015b) also known as Darwinian mimicry (Archila & Bertolini 2015a). Myophily in orchids is still quite neglected, with only few research papers published up to date (e.g. Borba & Semir 2001), including the studies of Pouyanian mimicry (pseudocopulation) in *Lepanthes* Sw. (Archila 2001).

According to Tremblay 1992, specialization to a specific pollinator is a common feature in the *Orchidaceae*. However, later studies suggested that this is an exception among angiosperms and gener-

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alized systems are more common (Ollerton 1996, Waser & al. 1996). Several observations revealed the low fidelity and low specificity between plant species and flies in many orchid groups (Christensen 1994), and few proved a more specific relationship between plants and their pollinators (e.g. Borba & Semir 1998).

2. Materials and methods

The material was collected at the "Estación Experimental de Orquídeas de la Familia Archila", Cobán (Guatemala), in December 2017. The fly adults were photographed and then taken alive directly from orchid flowers using a hand net. The specimens were preserved in a mixture of ethanol, glycerine and formaldehyde, and rinsed in 70 % and 30 % ethanol and in water to recover natural coloration. The microphotographs were made using a Leica M205A microscope and the LAS Montage multifocus. The dipteran specimens were identified using the keys by Burla 1956 and Grimaldi 1987.

3. Results and discussion

3.1. Pollinated orchids

Masdevallia Ruíz & Pav. is probably one of the most recognizable members of the *Pleurothallidinae*. The genus comprises about 350 species distributed from southern Mexico to southern Brazil (Luer 1986), with the highest diversity in the Andes of Colombia, Ecuador, Peru and Bolivia. The plants are caespitose and have fleshy, smooth leaves. The often brightly colored, large and showy flowers commonly have long-tailed sepals and obscure, small petals (Fig. 1).

During our studies on orchid biology carried out at the Estación Experimental de Orquídeas de la Familia Archila in Cobán (Guatemala), we observed fly pollination in two species of the genus *Masdevallia*, *M. floribunda* Lindl. and *M. tuerckheimii* Ames. Both species were found on the trunks of numerous trees located across the area of the Station (e.g. *Coffea arabica* L., *Citrus sinensis* (L.) Osbeck and *Eugenia paniculata* Jacq.). All of them were located in a rather shady area. Both *Masdevallia* species spread naturally across the Station. The flowering period of these two species ranges from June to February, coinciding with the rainy season.

Masdevallia floribunda (Fig. 1B) occurs typically on trees in moist to wet forests, at an elevation of about 900 to 1,200 m (Woolward & Lehmann 1896). Its distribution range extends from Mexico to Honduras. Plants are epiphytic, erect-spreading and clustered. The secondary stem is covered with short papery sheaths when young. The leaves are somewhat glossy, broadest above the middle, leathery, with glossy petiole, rounded at the apex and grooved along the midrib. Inflorescences are single-flowered, arising from the base of the leaf, spreading. The sepals are whitish, finely dotted and slightly tinged with crimson. The dorsal sepal is erect to reflexed, joined to the lateral sepals, and abruptly tapering into a cauda. The lateral sepals are fused, yellow at the base, with brownish-yellow to greenish, pointing-down to reflexed caudae. The petals are linear-oblong, white and toothed at the truncate tip. The lip is hinged to the column-foot, reflexed at tip, white with crimson dots and a yellow tip. The column is greenish with purple edges. The anther produces two obovoid pollinia.

Masdevallia tuerckheimii (Fig. 1D) occurs in wet lower mountain rain forest, at an altitude of c. 750–800 m. This species is locally abundant along creeks and in gallery forests, on shaded tree trunks. Its distribution range covers Mexico (Chiapas) and Guatemala. Plants of *M. tuerckheimii* are epiphytic, caespitose herbs. Their stems are terete, sulcate, very short and covered by two membranaceous sheaths. The leaf blades are convex, arcuate, oblanceolate-spatulate with the apex obtuse-subacute and the base attenuate into a sulcate petiole. Inflorescences in this species are short, pendulous and facultatively bifloral. If the first flower has been pollinated, the plant does not form the second one, but when the first flower fails, the second one develops. The sepals form a basal tube. The dorsal sepal is usually yellowish, slightly tinged with purple at the opening of the tube. The lateral sepals are usually pinkish or creamy-white with many clustered dots and/or short, transverse, purple stripes and yellow caudae. The petals are white, very similar to the lip which additionally is spotted with purple. The dorsal sepal is partially united with the lateral ones, rhombic, abruptly caudate. The lateral sepals are connate, forming an obovate to transversely elliptic-oblong blade, abruptly bicaudate. The petals are oblong, truncate, erose-dentate at the apex, with a marginal keel in the lower margin. The lip is short-clawed, oblong, with two plates separated by an axial groove, truncate-obtuse, deflexed, thickened and erose-dentate at the apex. The column is slender, subclavate, slightly arcuate, cream-white with a solid purple line on the ventral margins and the clinandrium. The two pollinia are ovoid-subclavate, compressed, with a small viscidium.

Masdevallia floribunda and *M. tuerckheimii* are often considered as conspecific. However, the latter species can be delimited by the larger, darker and more showily colored flowers (yellow caudae and densely purple-spotted sepals), less floriferous habit, and short, pendulous inflorescences. The appearance of the flowers of *M. tuerckheimii* is very different from that of *M. floribunda*. Clearly, *M. floribun-*

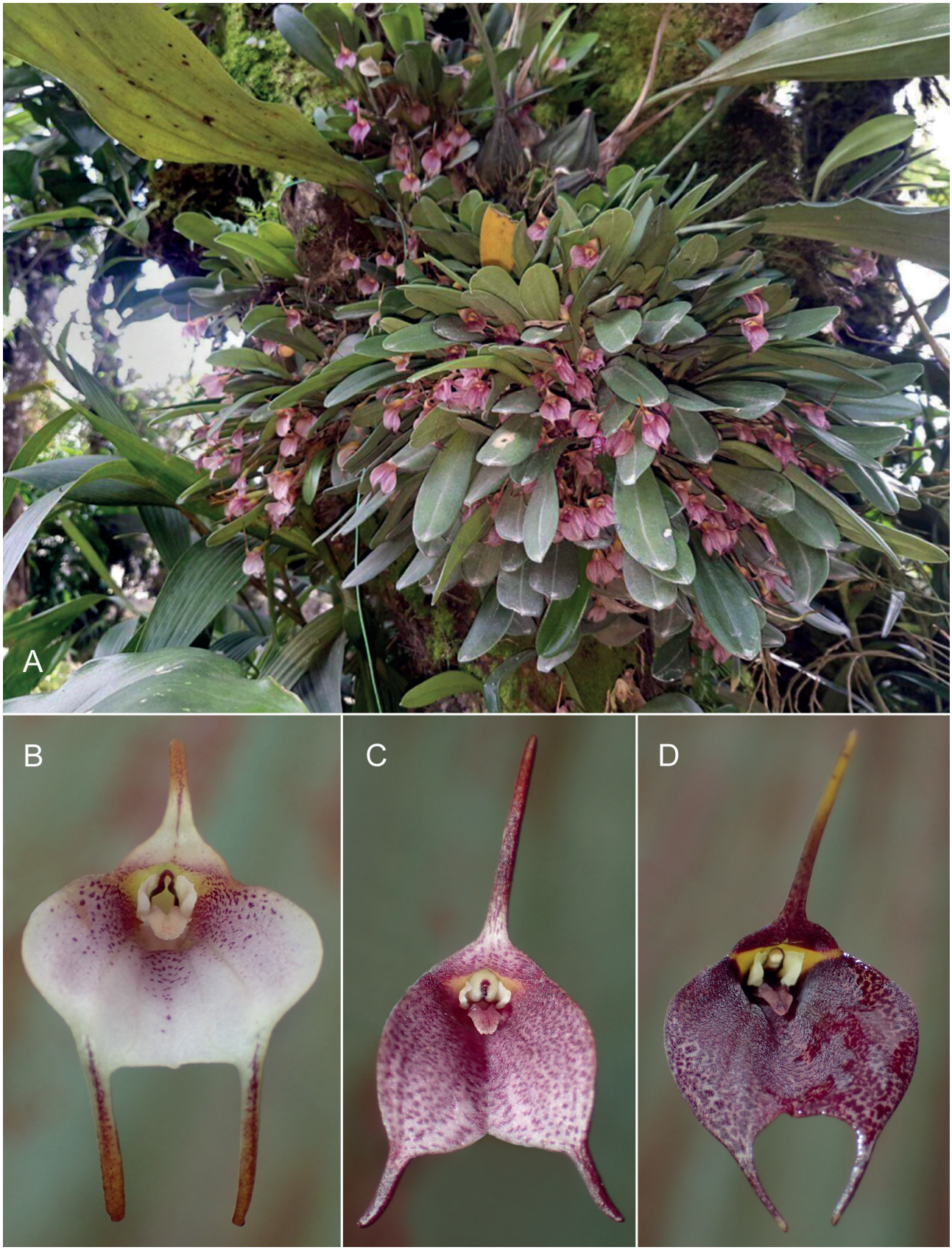

Fig. 1. *Masdevallia*. (A) Habit (phot. M. Veliz). – (B–D) Flowers. (B) *Masdevallia floribunda*, (C) hybrid of *M. floribunda* × *M. tuerckheimii*, (D) *M. tuerckheimii*.

da is a species of cloud forests and *M. tuerckheimii* is a species of tropical rain forests. However, there are ecotone zones between these two ecosystems where both species and some intermediate individuals (hybrids, Fig. 1C) can be found.

3.2. Pollinators

Further studies led us to the identification of the pollinators as representatives of the genus *Zygothrica* Wiedemann (*Diptera-Drosophilidae*). This species-rich genus is widely distributed in the Neotropical and Indopacific regions. As Grimaldi 1987 reports, swarms of these dipterans can be found above bracket fungi (e.g. *Polyporus tricholoma*) that grow from fallen rainforest trees in Latin America. However, the most prominent and principal hosts of *Zygothrica* seem to be bulky and fleshy flowers where the larvae may feed (Grimaldi 1987). It was indicated that members of ten angiosperm families are recorded as hosts of those florophagous dipterans: *Acanthaceae* Juss. (*Aphelandra* R. Br.), *Campanulaceae* Juss. (*Centropogon* C. Presl), *Lamiaceae* Martinov (cited as *Labiatae*; *Salvia* L.), *Fabaceae* Lindl. (cited as *Leguminosae*; *Erythrina* L.), *Liliaceae* Juss., *Marantaceae* R. Br. (*Calathea* G. Mey.), *Musaceae* Juss. (including *Heliconiaceae* Nakai), *Passifloraceae* Juss. ex Roussel (*Passiflora* L.), *Solanaceae* Juss. (*Brunfelsia* L., *Cestrum* L.), *Costaceae* Nakai (*Costus* L., *Dimerocostus* Kuntze), and *Zingiberaceae* Martinov (*Hedychium* J. Koenig). In terms of numbers of dipterans reared, the order *Zingiberales* Griseb. (*Heliconiaceae*, *Marantaceae*, *Musaceae*, *Zingiberaceae*) appears to be the most important host group (Grimaldi 1987). According to Endara & al. 2010, some *Orchidaceae* (*Dracula* Luer) can also be hosts for *Zygothrica* species.

The *Zygothrica* adults are defined by a prominent and sharp facial carina, a medially cleft oral margin, and a sclerotized proboscis with an acute joint between the mentum and submentum (Grimaldi 1987). Adult males of over a dozen of species have their heads broadened (hypercephaly), up to three times wider than the thorax - the body proportions being the most conspicuous character for *Zygothrica* (Fig. 2A, 2B). The presently examined *Zygothrica* adults belong to two, presumably undescribed species, thus they are tentatively named as *Zygothrica* spec. 1 and spec. 2. The distinct hypercephaly in the examined males, with a head/thorax width ratio of c. 1.5 and the transparent wing membrane lacking infuscations or markings (Fig. 2A, 2B) indicate that *Zygothrica* spec. 1 belongs to the *caudata* subgroup of the *dispar* group. Following the key by Burla 1956, *Zygothrica* spec. 2 keys out under *Z. mesopoeyi*, however, this species has so far only been recorded from SE Brazil and was defined as incertae sedis (group membership unknown) by Grimaldi 1987. The only relative that has been recorded that far north is *Z. poeyi* Sturtevant (the *atriangula* species group sensu Grimaldi 1987), though a color pattern on the abdomen of this species differs distinctly from that found in the presently examined specimens (Fig. 2C). Consequently, we recognize our specimens as *Zygothrica* spec. 2 aff. *mesopoeyi* Burla.

3.3. Pollination

Our investigation proves that *Masdevallia* species attract both males and females of *Zygothrica* and that both can carry the pollinia. According to our observations, they landed on the tepals and, after a few minutes of exploring the flower surface, they reached the lip region. The deposition of pollinia occurred when the fly entered the space between lip and column. The pollinia got attached to proximal abdominal tergites right behind the postnotum, between the wing bases, and protruded distinctly above the body from in between the wings, as shown in Fig. 3. On occasion several female and male individuals were observed at the same time on a single *Masdevallia* flower (Fig. 3A), which may suggest that flowers of these orchids are a place of habitual feeding and mating. The flies visited flowers regularly, from the early morning until dusk.

4. Conclusions

Myophilous flowers are relatively small, simple, actinomorphic, bright to dull colored (brown or green, often marked with dark spots or stripes) and with nectar produced in open shallow nectaries. The floral odor may be musky, slightly sweet and fruity, or malodorous (Endress 1994, Kite & al. 1998, Jürgens & al. 2006, Johnson & Jürgens 2010, Humeau & al. 2011, Davies & Stpiczyńska 2014, Stpiczyńska & al. 2015). Flowers of *Masdevallia floribunda* and *M. tuerckheimii* meet almost all those criteria. Moreover, following the suggestion by Grimaldi 1987, it may be that *M. floribunda* and *M. tuerckheimii* deceive their pollinators and, similarly to the closely related genus *Dracula* (e.g. Endara & al. 2010), mimic a fungus. Further research, including chemical and micromorphological analysis, is needed to find out how exactly those *Masdevallia* species attract their pollinators.

Coexistence of both orchid species in a relatively small area, together with sharing the same habitat, flowering time and pollinators, point towards possible hybridization and existence of natural hybrids between both examined *Masdevallia* species. The Estación Experimental de Orquídeas de la Familia Archila is located in a cloud forest, at an alti-

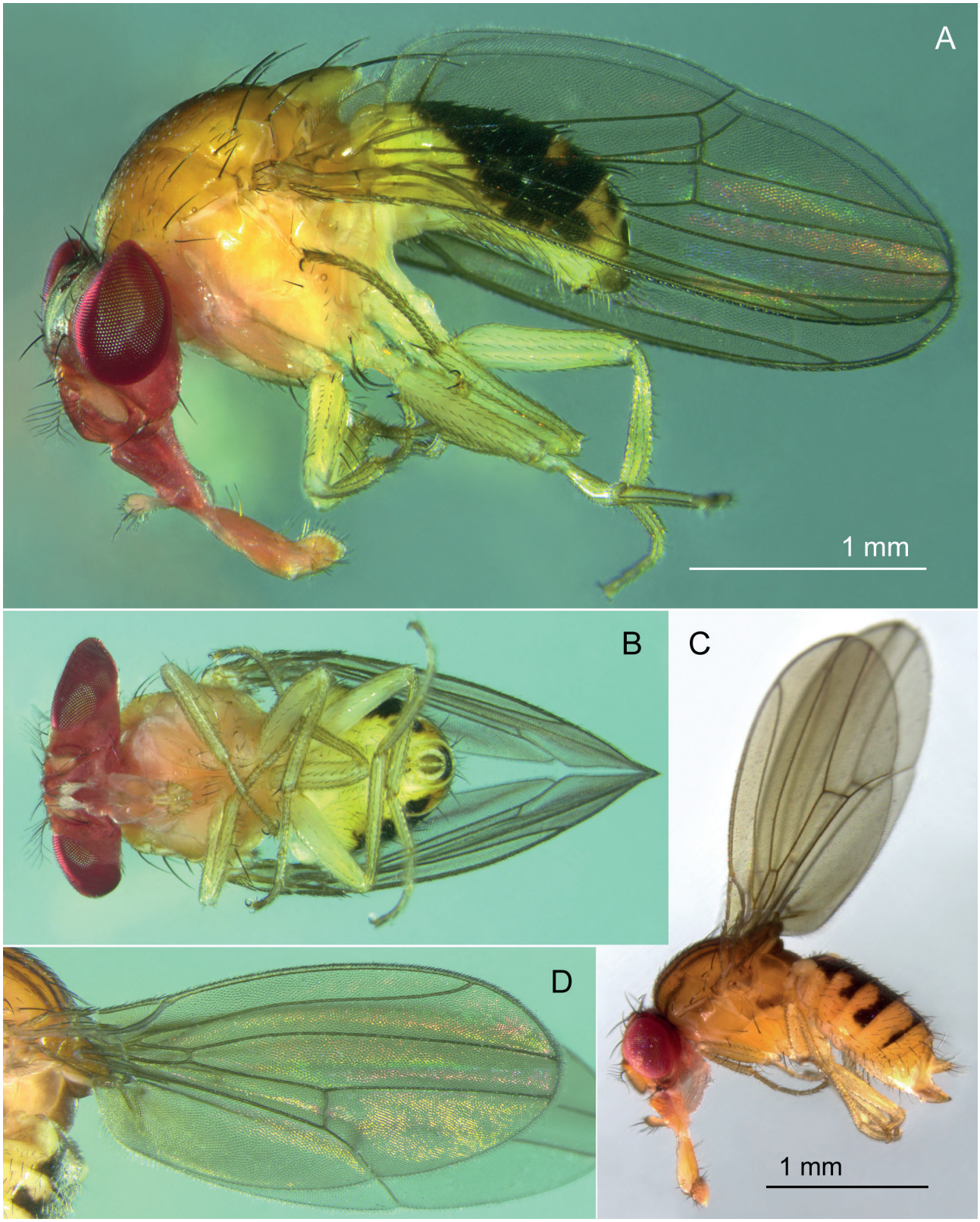

Fig. 2. *Zygothrica*. (A, B) *Zygothrica* spec. 1, hypercephalic male in lateral (A) and ventral view (B). – (C, D) *Zygothrica* spec. 2, female in lateral view (C) and its wing (D).

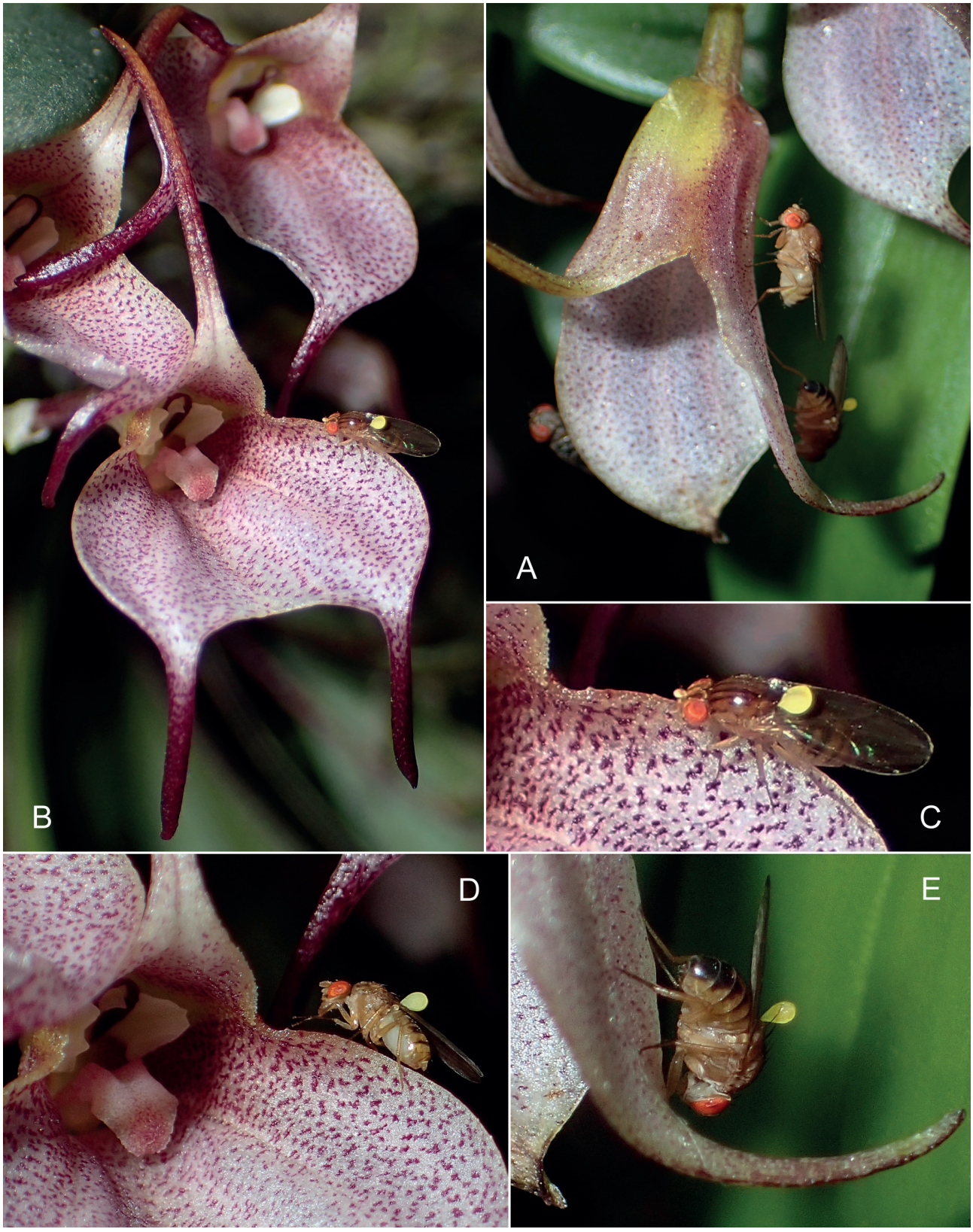

Fig 3. Adult *Zygothrica* specimens bearing *Masdevallia* pollinia (A–E).

tude of 1,320 m above sea level, from where plants of *M. tuerckheimii* were taken to the Station, closer to the *M. floribunda*. This apparently boosted the pollination activity of the *Zygothrica* adults on the flowers of both orchid species and effectively stimulated hybridization.

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References

Ackerman J. D. 1983. Specificity and mutual dependency of the orchid-euglossine bee interaction. – Bot. J. Linn. Soc. 20: 301–314.

Archila F. 2001. *Lepanthes* de Guatemala. Monografía del género *Lepanthes* Sw. (*Orchidaceae*) para Guatemala. – Editorial Kamar S.A.; Guatemala City. 281 pp.

Archila F. & Bertolini V. 2015a. *Hibiscorchis* un nuevo género de orquídea de la Isla de Borneo, con mimetismo seductivo. – Revista Guatemalensis 8(1): 6–14.

Archila F. & Bertolini V. 2015b. Elaiophores, a labellum anatomic structure, important in the pollination syndrome in *Pleurothallis* (*Orchidaceae*). – Revista Guatemalensis 8(1): 15–23.

Borba E. L. & Semir J. 1998. Wind-assisted fly pollination in three *Bulbophyllum* (*Orchidaceae*) species occurring in the Brazilian 'campos rupestres'. – Lindleyana 13: 203–218.

Borba E. L. & Semir J. 2001. Pollinator specificity and convergence in fly-pollinated *Pleurothallis* (*Orchidaceae*) species: a multiple population approach. – Ann. Bot. 88: 75–88.

Borg-Karlson A. K. 1987. Chemical basis for the relationship between *Ophrys* orchids and their pollinators: III. Volatile compounds of species in the *Ophrys* sections *Fuciflorae* and *Bombyliflorae* as insect mimetic attractants/excitants. – Chem. Scripta 27: 313–325.

Burla H. 1956. Die Drosophilidengattung *Zygothrica* und ihre Beziehung zur *Drosophila*-Untergattung *Hirtodrosophila*. – Mitt. zool. Mus. Berlin 32: 189–321.

Christensen D. E. 1994. Fly pollination in the *Orchidaceae*. – In: Arditti J. (ed.). Orchid biology: reviews and perspectives VI, p. 415–454. – Wiley; New York.

Davies K. L. & Stpiczyńska M. 2014. Labellar anatomy and secretion in *Bulbophyllum* Thouars (*Orchidaceae*: *Bulbophyllinae*) sect. *Racemosae* Benth. & Hook. f. – Ann. Bot. 114(5): 889–911.

Dressler R. L. 1968. Observations on orchids and Euglossine bees in Panama and Costa Rica. – Rev. Biol. Trop. 15: 143–183.

Endara L., Grimaldi D. A. & Roy B. A. 2010. Lord of the flies: pollination of *Dracula* orchids. – Lankesteriana 10(1): 1–11.

Endress P. K. 1994. Diversity and evolutionary biology of tropical flowers. – Cambridge University Press; Cambridge.

Grimaldi D. A. 1987. Phylogenetics and taxonomy of *Zygothrica* (*Diptera*, *Drosophilidae*). – Bull. Amer. Mus. Nat. Hist. 186: 103–268.

Humeau L., Micheneau C. & Jacquemyn H. 2011. Sapromyiophily in the native orchid, *Bulbophyllum variegatum*, on Réunion (Mascarene Archipelago, Indian Ocean). – J. Trop. Ecol. 27: 591–599.

Johnson S. D. & Jürgens A. 2010. Convergent evolution of carrion and fecal scent mimicry in fly-pollinated angiosperm flowers and a stinkhorn fungus. – S. African J. Bot. 76(4): 796–807.

Jürgens A., Dötterl S. & Meve U. 2006. The chemical nature of fetid floral odours in stapeliads (*Apocynaceae-Asclepiadoideae-Ceropegieae*). – New Phytol. 172: 452–468.

Kite G. C., Hetterscheid W. L. A., Lewis M. J., Boyce P. C., Ollerton J., Cocklin E., Diaz A. & Simmonds M. S. J. 1998. Inflorescence odours and pollinators of *Arum* and *Amorphophallus* (*Araceae*). In: Owens S. J. & Rudall P. J. (eds.). Reproductive Biology, p. 295–315. – Royal Botanic Gardens, Kew.

Luer C. A. 1986. Icones Pleurothallidinarum 2. Systematics of *Masdevallia* (*Orchidaceae*). – Monogr. Syst. Bot. Missouri Bot. Gard. 105: 1–300.

Ollerton J. 1996. Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. – J. Ecol. 84: 767–769.

Paulus H. F. & Gack C. 1990. Pollination of *Ophrys* (*Orchidaceae*) in Cyprus. – Pl. Syst. Evol. 169: 177–207.

Stpiczyńska M., Davies K. L. & Kamińska M. 2015. Diverse labellar secretions in African *Bulbophyllum* (*Orchidaceae*: *Bulbophyllinae*) sections *Ptiloglossum*, *Oreonastes*, and *Megaclinium*. – Bot. J. Linn. Soc. 179(2): 266–287.

Tremblay R. L. 1992. Trends in the pollination ecology of the *Orchidaceae*: evolution and systematics. – Canad. J. Bot. 70: 642–650.

van der Pijl L. & Dodson C. H. 1966. Orchid flowers: their pollination and evolution. – University of Miami Press; Coral Gables, Miami.

Waser N. M., Chittka L., Price M. V., Williams N. M. & Ollerton J. 1996. Generalization in pollination systems, and why it matters. – Ecology 77: 1043–1060.

Williams N. H. & Dodson C. H. 1972. Selective attraction of male euglossine bees to orchid floral fragrances and its importance in long distance pollen flow. – Evolution 26: 84–95.

Woolward F. H. & Lehmann F. C. 1896. The Genus *Masdevallia*. – R. H. Porter; London.

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