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Fruit-breeding drosophilids (Diptera) in the Neotropics: playing the field and specializing in generalism?

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Running title: Fruit-breeding Drosophilids in the Neotropics

Abstract.

1. Species of Drosophilidae are frequently used as model organisms, but their relationships with the environment, particularly at immature stages, remain poorly known.
2. This is the most comprehensive survey to date of fruit-breeding drosophilids and their hosts in the Neotropics. We analyze drosophilid host-utilization patterns as to geographical origin (native versus exotic) and level of specialization.
3. The 180 species of plants recorded as drosophilid hosts are distributed across the main Angiosperm lineages and fleshy-fruited orders; plant families that hosted the most drosophilid species were Arecaceae, Moraceae, and Myrtaceae. The 100 nominal drosophilid species recorded breeding in fruits belong to just over one third of Neotropical genera; most species (91) belong to *Drosophila*. Drosophilid species with the greatest resource breadth were *Drosophila simulans*, *D. nebulosa*, and *Zaprionus indianus*.
4. Exotic drosophilids breed in more plant species than Neotropical drosophilids and use exotic hosts more frequently, possibly because they are generalists that have survived the trial of introduction and establishment in the Neotropics. Native drosophilids are more variable in resource breadth and sometimes adopt exotic hosts.
5. Amongst the 49 drosophilids with enough records for analysis (>4), 48 were categorized as generalists. One possible explanation for such overwhelming generalism is the high diversity of Neotropical habitat or hosts. A second, non-exclusive explanation, suggested by recent studies, and empirically supported by the absence of host specialization found in this study, is that drosophilids could be selective of the dominant yeasts and bacteria in host tissue, and not of the hosts themselves.

Key words: breeding site, *Drosophila*, hosts, Moraceae, Myrtaceae, niche breadth.

1 **Introduction**

2
3 Drosophilidae currently includes about 4400 species distributed globally (Bächli, 2018).
4 *Drosophila melanogaster* is a familiar genetic model, but over the course of the past century
5 many other drosophilid species have been used to study cellular, developmental, ecological and
6 evolutionary processes (Markow & O'Grady, 2008). Relating poorly known natural
7 environmental drivers of drosophilid evolution to detailed knowledge of their development,
8 micro-evolution and genetics, derived from laboratory studies, is essential for understanding
9 the processes and patterns of their evolution (Powell, 1997). Drosophilids breed and feed in
10 decomposing organic matter, such as fallen fruits, flowers, and fungi (Carson, 1971). Adults
11 often feed on a wide range of substrates, whereas immature stages are nutritionally more
12 demanding and require many vitamins and minerals to develop until eclosion (Sang, 1978).
13 Drosophilid larvae have a strong dispersal limitation because they are restricted to the resource
14 patch chosen by the female during oviposition (Heard & Remer, 1997). Female choice, in turn,
15 is based on a simple decision-making process that takes in account the odors released by
16 potential resources during the decaying process (Yang *et al.*, 2008). The nutrition of larvae and
17 adults is obtained from the rich microbiota, especially yeasts and bacteria, associated with
18 rotting substrata (Chandler *et al.*, 2011, 2012). Through decomposition, microbes make host
19 chemicals available to the drosophilid, and may detoxify otherwise harmful plant compounds
20 (Starmer, 1981).

21 Sporadic collection by nineteenth-century European naturalists began the study of
22 Neotropical drosophilids. The first ecological studies took place during the 1940s (Dobzhansky
23 & Pavan, 1950; Pavan, 1959), and, since the 1960s, drosophilids have been investigated by
24 many primarily South American research groups (Pipkin, 1965; Sene *et al.*, 1980; Budnik &
25 Brncic, 1983; Hoenigsberg, 1995; Goñi *et al.*, 1998; Rafael & Vela, 2000; Martins, 2001;

1 Klaczko, 2006; Manfrin & Sene, 2006; Tidon, 2006; De Toni *et al.*, 2007; Beltrami *et al.*, 2010;
2 Soto *et al.*, 2011). The first systematic revision on drosophilids distributed in the Neotropical
3 region was published in the early 1980s (Val, 1981), and currently there are about 900
4 drosophilid species (32 genera) recorded from the Neotropics. Cosmopolitan *Drosophila* is the
5 most species-rich genus (442 species), followed by *Cladochaeta* (116 species) and *Zygothrica*
6 (106 species) (Brake & Bachli, 2008).

7 Estimates of Neotropical flowering plant species richness oscillate between 90,000 and
8 110,000, representing approximately 37% of global flowering plant diversity (Antonelli &
9 Sanmartin, 2011). Species with fleshy, endozoochorous fruits are dominant in the woody strata
10 of many tropical habitats. In the Atlantic forest of eastern Brazil, 45-90% of woody species are
11 endozoochorous (Almeida-Neto *et al.*, 2008) and in the savannas of central Brazil
12 approximately 70% of trees have fleshy fruits (Kuhlmann & Ribeiro, 2016a). Most fleshy fruits
13 are highly constrained to attract their vertebrate dispersers by producing powerful visual and
14 olfactory signals, that may also act as environmental cues for female fruit flies (Clarke, 2016).
15 Fruits have long been recognized as the prime breeding sites for tropical drosophilids (Carson,
16 1971), but there is no review of their breeding sites in the Neotropics. There are reviews of
17 breeding site records for tropical Africa (Lachaise & Tsacas, 1983), eastern North America
18 (Carson & Stalker, 1951), Hawaii (Magnacca *et al.*, 2008), Japan (Kimura *et al.*, 1977), and for
19 temperate (Shorrocks, 1982) and domestic drosophilids (Atkinson & Shorrocks, 1977). In this
20 study, we provide a broad review of fruit-breeding drosophilids in the Neotropics. Data was
21 collected from various sources and used to explore the phylogenetic spread of hosting plant
22 taxa, host-utilization patterns according to geographical origin (native versus exotic), and to
23 classify drosophilids into generalists or specialists based on breadth of host use.

24 25 **Data collection and analysis**

Records of drosophilids using fruits as breeding sites in the Neotropics were obtained from three sources: (1) published scientific papers; (2) thesis and dissertation databanks; and (3) unpublished data collected between 1999 and 2013. Delimitation of the Neotropics followed the division proposed by the BioSystematic Database of World Diptera (Evenhuis *et al.*, 2008), which includes South America and most of Central America, but excludes some Mexican states. Nearest-neighbour distances (Clark and Evans, 1954; Davis, 1986) were used to test under- and over-dispersion of sample points, with Donnelly edge correction. We exhaustively searched digital databases (Web of Science, Drosophila Information Service, Flybase and the Brazilian Digital Library of Thesis and Dissertations- BDTD), using the key words [“drosophil*” and “breeding-sites”], and [“drosophil*” and “fruits”], up to December 2018. We also consulted the references in the papers recovered. This search resulted in 41 papers and one unpublished doctoral dissertation recording drosophilids breeding in fruits in the Neotropics. These references are listed at the bottom of Table 1. Drosophilids from the unpublished data mentioned in (3) were identified by the first and last authors, both of whom are experienced in drosophilid taxonomy (Tidon, 2006; Valadão *et al.*, 2010). Plant species in this category were identified by the second and third authors, both of whom are experienced in the central Brazilian flora (Proença *et al.*, 2006; Kuhlmann & Ribeiro, 2016b). Voucher material is deposited in the Universidade de Brasília entomological collection and herbarium (UB herbarium, M.P. Kuhlmann collections).

The database only includes studies that recorded the use of fruits as breeding sites for drosophilid larval stages. Thus, data were discarded from baited traps and adults collected from resources using nets or entomological vacuums. In studies with more than one collection procedure, only data from collected fruits were considered. Finally, only data from fruits collected near the host species were used; fruit from markets and waste containers were

1 excluded. Consequently, each breeding record reports the development of the larval stage of a
2 drosophilid species, associated with a plant species from a single reference source.

3 Drosophilid names were checked in the World Catalogue of Drosophilidae (Brake &
4 Bächli, 2008) and plant names were checked in The Plant List (2010). One drosophilid name,
5 *Drosophila fumocalloptera*, is a *nomen nudum* and was removed from our database. Eight
6 drosophilid species with dubious identifications (*affinis*) were retained in the database but
7 removed from the analysis. In some references, drosophilids were identified only at group or
8 generic level, e.g., *willistoni* and *tripunctata* groups. A drosophilid species was considered
9 neotropical if its distribution is restricted to this biogeographical region, or if it belongs to a
10 species group endemic to the Neotropics. The geographical distribution and authorities for
11 drosophilid species was checked in Taxodros (Bächli, 2018). Plant order and family
12 circumscription followed the Angiosperm Phylogeny Group IV (APG IV, 2016) and Stevens
13 (2012). Some references did not provide scientific plant names. Where common names are well
14 known and associated to other data, such as the geographic area, it was often possible to infer
15 plant species identity with a high level of confidence but, in some cases, these could only be
16 determined to generic or familial levels. Plants determined to genus only were considered
17 Neotropical if the genus is endemic to the Neotropics; for pantropical or cosmopolitan genera,
18 they were classed as unknown. Geographical provenance of plant genera and species were
19 obtained from Mabberley (1997) and The Plant List (2010).

20 All plant and drosophilid nominal species were classified regarding their geographical
21 origin, as native or exotic (non-Neotropical), and a matrix was built to show associations among
22 these taxa. The proportion of positive associations observed between neotropical (N) and exotic
23 (E) fruit-bearing hosts (F) and drosophilids (D) was then calculated for the four possible pairs:
24 NF x ND, NF x ED, EF x ND and EF x ED. The expected percentage of associations for each
25 pair was predicted based on the total possible associations of the matrix. The adherence between

observed and predicted association percentages in each category was tested using the Chi-square Goodness of fit test followed by an Exact Binomial test for each pair as a post-hoc test (Zar, 1999). For this analysis, we only used nominal species.

Classification of drosophilids as generalists or specialists was based on Magnacca *et al.* (2008), where a species was considered a specialist if two conditions were met. Firstly, at least two thirds of its breeding records are associated to a single plant family. Secondly, any other family has less than 25% of the remaining records, assuring a clear preference for a single family. For example, a species with 60 breeding records would be considered a specialist if at least 40 records were made in a single plant family, and any other family had no more than 15 records. Thus, a drosophilid may be considered as a specialist even if it uses alternative plant families as secondary or occasional hosts. This analysis was conducted only for nominal species recorded at least four times, since low sampling could inflate the number of specialists. All statistical analyses were performed using R 2.14.1 software (R Development Core Team, 2011).

The phylogenetic tree for Drosophilidae presented is an adaptation of Yassin (2013) since his phylogeny included all of the genera and all of the groups we recorded except for one, *Drosophila* group *caponei* that is phylogenetically unplaced (Bächli, 2018) and, as far as we are aware, has never been sampled in a phylogeny. This was complemented by that of Russo *et al.* (2013) since the latter included many Neotropical species not available in Yassin (2013). Highly supported (posterior probabilities >.9), monophyletic lineages not recorded in our study were left in the phylogeny but had their branches collapsed to aid visualization. Drosophilids identified as aff. were not included. When the Yassin (2013) phylogeny included one or more species of a monophyletic group that was the same as ours, but sampled different species to that which we recorded as a fruit-breeder, the genus name was left and our species was placed alongside it with the symbol “~” to indicate that the phylogenetic position is tentative and follows Bächli (2018).

Fruit-breeding drosophilids and their hosts in the Neotropics

Eighty-eight sites were recorded in 10 countries: Argentina (1), Brazil (42), Chile (6), Colombia (12), Costa Rica (1), Cuba (1), El Salvador (11), Honduras (1), Panamá (2) and Uruguay (11). Altitudes varied between sea-level to 2133 m.s.m. Sixty-nine of the 88 sites had unambiguous habitat information: 64% of these were recorded as natural habitats and 36% as man-made habitats. Three quarters of the natural habitat sites were forests, and most of these (20 sites) were lowland tropical rainforests or gallery forests (mostly <300m), widely ranging from ca. 15 ° N (Lancetilla, Honduras) to 27 ° S (Tucumán, Argentina); five other forest sites were at mid-altitudes (423-930 m), described as seasonal, subtropical or semi-deciduous forests, and three were above 1100 m, described as highland, gallery or cloud forest. Other natural areas (eight sites) were described as fields, prairies, wetlands, marshes, semi-desert forest (*caatinga*), savanas (*cerrados*), volcanic cliffs (*barrancos*) or simply as natural vegetation; these were mostly at mid-altitudes. The man-made habitats were plantations, gardens, orchards or urban areas and were mostly at low altitudes. All sample locations together were significantly under-dispersed ($R = 0.280$; $p < 0.0001$) and fell into two groups, one north and one south of the Amazon Basin. When the two groups were treated separately, both were also significantly under-dispersed ($R = 0.788$; $p < 0.0483$ and $R = 0.476$; $p < 0.0001$, respectively). However, in our opinion this bias does not undermine the results because, as shown above, the pool of sampled sites are geographically widely-distributed and cover a wide spectrum of ecological drivers that might be affecting drosophilid-fruit interactions such as altitude, climate, and vegetation type.

One hundred nominal species of drosophilids were recorded in this survey and one hundred and eighty hosting plant species. Nominal drosophilids were classified in eight genera:

1 *Chymomyza*, *Diathoneura*, *Drosophila*, *Hirtodrosophila*, *Neotanygastrella*,
 2 *Rhinoleucophenga*, *Scaptodrosophila* and *Zaprionus*, along with undetermined species in the
 3 genera *Drosophila*, *Mycodrosophila*, *Scaptomyza* and *Zygothrica* (Table 1). The most species-
 4 rich genus was *Drosophila* (91 species), followed by *Diathoneura* (3 species); other genera
 5 were represented by one species each. Hosting plants were recorded in one Gymnosperm family
 6 (Ginkgoaceae) and 49 Angiosperm families distributed throughout the major lineages (Table 2,
 7 Fig 1). One hundred and thirty-one plant hosts were classified at the species level, 48 at the
 8 genus level and one at the family level. New hosting plant taxa for the Neotropics recorded
 9 were 15 species, six genera, two families (marked in Table 2 in **bold**) and one order
 10 (Celastrales). The plant families with the most species recorded as used by drosophilids were
 11 Myrtaceae, Moraceae, Arecaceae and Rosaceae (Fig 2); those with the most diverse use as a
 12 resource at the drosophilid species level were Arecaceae (59 drosophilid species), Moraceae
 13 (37), Myrtaceae (34), Solanaceae (32) and Rutaceae (29). Although these records suffer from
 14 problems inherent to large compilations, such as partially identified taxa, a sampling bias that
 15 favors easy to find cultivated plants, and unequal effort expended on different potential host
 16 plants or geographical areas (Jorge *et al.*, 2017) general patterns have emerged.

17 Drosophilids were recovered both from true, fleshy fruits (mature developed ovaries),
 18 and from non-ovarian fleshy tissues associated with seed dispersal such as sarcotesta (*Ginkgo*;
 19 Ginkgoaceae), receptacle (*Fragaria*; Rosaceae), hypanthium (*Malus*; Rosaceae), peduncle
 20 (*Anacardium*; Anacardiaceae) and infructescence axis (*Hovenia*; Rhamnaceae). The dominance
 21 of species that provide fleshy tissue is probably because they provide a rapid growth medium
 22 for yeasts and bacteria due to the high percentages of water and sugars, offering suitable
 23 conditions and resources for larval development. Although Neotropical drosophilids breed on
 24 small, dry, rotting fallen fruits, these are particularly vulnerable to desiccation and therefore are
 25 effectively colonized only in the wet season (Mata *et al.*, 2015). Fleshy fruits, on the other hand,

retain minimum quality and are suitable as breeding sites at all times of the year (Pipkin, 1965; Leão & Tidon, 2004).

Host-utilization patterns

There was no obvious phylogenetic structuring (i.e., as detected by visual inspection of the tree; no tests were performed) in the host plants used by drosophilids, but all major Angiosperm lineages were represented (Fig 1). Host angiosperm families were distributed throughout the Magnoliid, Monocotyledon, Rosid and Asterid lineages (Fig 1). About half of all possible orders had records of one or more host species; most (but not all) non-hosting orders were non-Neotropical, species poor (>100 species in the Neotropics, or predominantly to invariably dry-fruited. Several orders were “conspicuous by their absence”, i.e., host species were expected since they are fleshy-fruited and diverse or common in the Neotropics. In the Magnoliid lineage, order Piperales (the black pepper, *Piper nigrum* L., order) was the unexpected exception. The lack of records in Piperales may be related to their small, unattractive fruits, or due to their alkaloidal chemical defenses; the order is rich in piperamides. Piperamides in the young fruits of two Neotropical species of *Piper* (Piperaceae) inhibited yeast growth (Regasini *et al.*, 2009). Gottschalk (2008) recorded negative results for neotropical *Peperomia* (Piperaceae) fruits. In Hawaii, native *Peperomia* and exotic *Piper nigrum* L. were reported as rare hosts but only rotting leaves or stems were used (Magnacca *et al.*, 2008). In the Monocotyledon lineage, there were no unexpected exceptions at order level and family Arecaceae (palms) was noteworthy as one of the richest resources, cited in almost half of the studies, from which 59% of all drosophilid species were found to emerge. The basal Eudicotyledon order Ranunculales (the buttercup, *Ranunculus bulbosus* L. order) was another unexpected exception. In the Rosid lineage, there were no unexpected exceptions at order level and it is worth noting that it accounted for about half of host families (26; 51%) and host species

(89; 49%). Furthermore, the second and third most used families, Moraceae and Myrtaceae, are Rosids. In the Asterid lineage, unexpected exceptions were the Fagales (*Morella*, Myricaceae has berries), Saxifragales (*Ribes*, Grossulariaceae has berries), Boraginales (*Cordia* and *Tournefortia*, Boraginaceae, have drupaceous fruits) and the Dipsacales (*Sambucus* and *Viburnum*, Adoxaceae, have drupaceous fruits). In temperate regions, *Ribes uva-crispa* L., *Sambucus nigra* L. and *Viburnum davidii* Franch. have been recorded as hosts of *Drosophila suzukii* (Grassi *et al.*, 2011; Lee *et al.*, 2015).

Three plant families (Arecaceae, Myrtaceae and Moraceae) were disproportionally represented amongst host plant species (40% of the total breeding records) and showed the highest richness of drosophilids. Arecaceae and Myrtaceae (but not Moraceae) are frequently cited as ecologically dominant in forests and savannas and all three families only produce fleshy fruits in the Neotropics, while other ecologically-important families (e.g., Asteraceae, Apocynaceae, Bignoniaceae, Fabaceae and Rubiaceae) produce mostly dry fruits or both types (van Roosmalen, 1985; Lughadha & Proença, 1996; Oliveira-Filho & Fontes, 2000; Françoso *et al.*, 2016). Staggemeier *et al.* (2016) found hyper-dominance of fruit pulp production by Arecaceae and Myrtaceae in a Brazilian Atlantic Forest community with 37 plant families; 72% of annual fruit pulp was produced by these two families alone.

Host utilization patterns: neotropical versus exotic species

The matrix between nominal drosophilids–plant hosts (Supporting Information, SI) showed that 6.6% of all possible associations were recorded. All four categories of neotropical/exotic drosophilid/plant (ND x NP, ND x EP, ED x NP and ED x ED) differed significantly from the rest of the matrix as to percentage of cell occupancy (Table 3) ($\chi^2 = 337.29$, $df = 3$, $p < 0.0001$). We used the value of 6.6% as the random expected occupancy for each category. Exotic drosophilids (coloured red in Supporting Information 1) showed higher

1 than expected cell occupancy, both on exotic and Neotropical plant hosts. Neotropical
2 drosophilids (coloured green in Supporting Information 1), on the other hand, showed lower
3 than expected cell occupancy, both on Neotropical and exotic plant hosts.

4 Why are exotic drosophilids more successful in exploring fruits than their neotropical
5 counterparts? First, exotic species are probably a subset: the ones that have survived the trial of
6 introduction into the Neotropics and successful establishment in a new environment.
7 Afrotropical *Drosophila simulans* and *Zaprionus indianus* currently dominate many
8 neotropical assemblages (Sene *et al.*, 1980, Tidon *et al.*, 2003, Döge *et al.*, 2015). In contrast,
9 Neotropical drosophilids are a mélange of species with varying degrees of sensitivity to
10 environmental heterogeneity. Moreover, exotic species may be homogenizing the microbiota
11 composition of rotting fruits in a way that favors their own offspring. Drosophilids can inoculate
12 microbes at their breeding sites through their faecal deposits and oviposited eggs (Bakula, 1969;
13 Gilbert, 1980), enhancing the resources available to hatching larvae. This rationale is consistent
14 with the Niche Construction Theory, which explains how organisms' niche modifications may
15 feedback to affect their ecological and evolutionary trajectories (Laland *et al.*, 1999; Buser *et*
16 *al.*, 2014). Anecdotally, one of the authors (RT) has recorded *Z. indianus* insistently chasing
17 Neotropical *D. nebulosa* away from a fallen mango (*Mangifera indica* L.) (Supporting
18 Information 2). Following the predictions of Biological Invasion Theory, exotic drosophilids
19 may have negative impacts on breeding success of native species (Vitousek *et al.*, 1996).

20 Although the lowest cell occupancy was of native drosophilids on exotic fruits (coloured
21 yellow in Supporting Information 1), some native drosophilid species do appear to be
22 successfully exploiting exotic fruits. The high floristic diversity of the Neotropics means that
23 exotic plant hosts often have native relatives at family or genus level; theoretically it should be
24 easier for a fruit-breeding drosophilid to adopt a new host that is phylogenetically close to an

original host. Furthermore, some exotic hosts such as mangos, citrus fruits and grapes have been cultivated in the Neotropics for over 300 years (Harris *et al.*, 2016).

Breadth of host use: drosophilids as generalists at plant family level

Only 49 of the 101 nominal drosophilid species were recorded more than four times, and all of these were categorized as generalists, except for *Drosophila subobscura*, a specialist in Rosaceae and an occasional user of Moraceae (both families are members of Order Rosales). The species with the greatest resource breadth were: *Drosophila simulans* (90 plant taxa), *D. nebulosa* (57), *Zaprionus indianus* (44), *D. willistoni* (43), *D. melanogaster* and *D. polymorpha* (both 42). Several studies did not completely discriminate between sibling species, particularly in the *Drosophila willistoni* subgroup (*D. willistoni*, *D. paulistorum*, *D. equinoxialis* and *D. tropicalis*), but when identifications were made, *D. willistoni* was usually the most common (Spassky *et al.*, 1971, Garcia *et al.*, 2014; Roque *et al.* 2017). If *D. willistoni* is assumed present in all references where the identification was incomplete, the number of plant species used by this drosophilid would be 114, the broadest niche breadth of all species reported.

It is noteworthy that when only exotic nominal species (12 species) are considered, 83% of the species were recorded as breeding in six or more plant orders, and 58% breeding in 10 or more plant orders; for native species, 31 % were recorded breeding in six or more plant orders, and 15% breeding in 10 or more plant orders (Table 1). When these species are plotted on the Drosophilid phylogeny - adapted from Yassin (2013) and Russo et al. (2013) – the wide-spectrum species (arbitrarily defined as breeding in 10 orders or more) appear concentrated in three clades: sister clades A and B that together form *Drosophila* subgenus *Sophophora* (also including *Lordiphosa*) and clade C. Within clade C, wide-spectrum species appear to be concentrated in a single subclade. Two exotic wide-spectrum drosophilid species are found out

1 of these three clades: *D. immigrans* and *Zaprionus indianus*. Clade D had no wide-spectrum
2 species.

3 The groups with the broadest diet breadth were *willistoni* (114 plant taxa), *melanogaster*
4 (104), *tripunctata* (89), *cardini* (76), *repleta* (57) and *guarani* (50). Each of these groups was
5 found in at least 30% of all resources sampled, showing great ecological versatility in breeding
6 sites.

7 Specialization theory states that the evolution of generalists is associated to variable
8 conditions, in which resources are unpredictable and hard to find in space and time, whilst the
9 evolution of specialists is favored by low environmental diversity, such as few, stable,
10 predictable resources (Levins & MacArthur, 1969; Futuyma & Moreno, 1988; Kassen, 2002).
11 When resource availability imposes distinct limitations on the same species of drosophilid, the
12 adaptive value of that species will be the weighted average of its performance under different
13 conditions (Kassen, 2002). Only rarely will a generalist species be most efficient in the way it
14 exploits any individual resource (see Remold, 2012 for exceptions), but its ability to exploit
15 many resources enhances its average adaptive value in spatially or seasonally heterogeneous
16 environments.

17 In this study, the criteria used for defining specialist species were permissive (the
18 analysis was at botanical family with incidental resource use allowed) in spite of which, all but
19 one drosophilid species were classified as generalists. Using the same criteria, Magnacca *et al.*
20 (2008) classified 67% of drosophilid species in Hawaii as specialists. Although the percentage
21 of specialists in their study fell nearly 10% when the number of required samples were increased
22 from >2 to >10, it remains that specialization in the Neotropical fruit-breeding drosophilids is
23 very low when compared to Hawaiian drosophilids. However, our results are not directly
24 comparable with Magnacca *et al.* (2008), since they included the whole Hawaiian drosophilid
25 fauna, which is dominated by leaf breeders (80%). Thirty-six species of Hawaiian drosophilids

1 use fruits to breed; applying the Magnacca criteria (>2 records) to these, only nine species
2 qualify, of which only one is a specialist (11%) (*Drosophila adiastrata* on Campanulaceae); the
3 rest are generalists. If the same criteria are applied but with a stricter threshold (>4 records),
4 only three species qualify, which are all generalists. Therefore, specialization amongst fruit-
5 breeders in Hawaii appears similar to the Neotropics, and low when compared to the high
6 degree of specialization (67%) found in the whole archipelago's drosophilid fauna. This could
7 either be associated to dominant leaf breeding (80% of Hawaiian drosophilids are leaf breeders)
8 or to their very restricted cloud forest habitat. Chemical and mechanical defenses in leaf tissue
9 vary widely between different plant groups and are known to affect the rate of litter
10 decomposition (Grime et al., 1996).

11 It is tempting to speculate that the use of different substrates (fruit x leaves) is the cause
12 of different levels of specialization between the Hawaiian drosophilid fauna and the Neotropical
13 drosophilid fruit breeders. In a systematic large-scale field survey of potential larval hosts in
14 Australia, Van Klinken and Walter (2001) found that 25 of the 43 surveyed species emerged
15 from a single host type (fruit or fungus). Chandler *et al.* (2011, 2012) showed that different
16 substrates (fruit, flower, fungus or cactus) are dominated by different microbial communities:
17 *Hanseniaspora* Berkhout is the dominant yeast genus associated with fruit-breeding
18 drosophilids, *Candida* Berkhout predominates amongst mushroom-breeding drosophilids and
19 *Pichia* Hansen amongst cactus-breeding drosophilids. Different drosophilid species show
20 preferences for different yeast species (Starmer & Fogleman, 1986), and yeast odors supposedly
21 supply the critical signal to establish the drosophilid–yeast–fruit relationship (Becher *et al.*,
22 2012). Furthermore, even when colonizing the same substrate drosophilids have differential
23 yeast preferences (da Cunha *et al.*, 1951; Morais *et al.*, 1992; Batista *et al.*, 2017). For some
24 cactophilic species of *Drosophila*, resource selection and oviposition appear to be largely
25 determined by interactions between the cactus species and its microbial community so that in

1 the presence of some yeasts, drosophilid species strongly prefer their primary hosts, while the
2 presence of other yeasts obliterate such differences in preference (Soto *et al.*, 2017). Such
3 studies suggest drosophilid specialization may occur at the microbiome level.

4 Other possible explanations for this discrepancy is that the large area of the Neotropics
5 results in more habitats and a higher climatic diversity, when compared to the relatively small
6 (although altitudinally diverse) Hawaiian Islands. High Neotropical floristic diversity, in
7 contrast to the low Hawaiian floristic diversity, can also contribute to resource unpredictability
8 and favor generalist drosophilids. A study in several Hawaiian montane rainforest plots of 0.2
9 ha (total area of 1.6 ha) recorded 59 vascular plant species, of which 11 were trees or lianas
10 (Kitayama *et al.*, 1995). In contrast, Neotropical forest plots of 0.1 ha across seven South
11 American countries recorded an average of 128 (± 64) trees, lianas and hemiepiphytes per plot
12 (110 plots from Argentina, Bolivia, Brazil, Chile, Colombia, Peru, and Venezuela; Phillips &
13 Miller, 2002). In the savannas of Central Brazil, the number of vascular plant species recorded
14 in one 0.1 ha plot was 230-250 (Silberbauer-Gottsberger & Eiten, 1983), and an average of 60
15 (± 25) woody species per site has been suggested (compiled from 625 sites; Franoso *et al.*,
16 2016), while the herbaceous layer has on average 5.6 times as many species as the woody layer
17 (Mendona *et al.*, 2008). Consequently, the average distance a host-specialist drosophilid has
18 to travel to find a specific host is probably much greater in most Neotropical habitats than in
19 most Hawaiian habitats. It is congruent with specialization theory that high floristic diversity
20 has favored a generalist strategy in Neotropical drosophilids, i.e., ‘the position and breadth of
21 the niche evolves to match the amount of environmental variation’ (Kassen, 2002, pg. 185).

22 Our results strongly contrast to what has been found for herbivores, generally known
23 for a narrow diet breadth (Jaenike, 1990; Lewinshon & Roslin, 2008). A global dataset recently
24 analysed by Forister *et al.* (2015) shows that most insect species are associated with a single
25 plant family, at least locally. A strong bias towards specialisation was also found in Asteraceae-

flowerhead endophagous assemblages analysed by an innovative framework that takes phylogeny, commonness and co-occurrence between resources and consumers into account (Jorge *et al.*, 2014). According to Loxdale and Harvey (2016), the concept of generalism is sensitive and may be illusory; even if species are polyphagous, their range of food items may still be highly selective due to genetic and phenotypic constraints. The generalism of insects, however, is not absolute: many species of tephritid fruit flies (*Bactrocera* spp.) utilize larval hosts across two to >20 plant families and are classified as generalists (Clarke, 2016). There may also be a problem distinguishing true generalists (i.e. multiple resource using species from a single site) from polyspecialists (i.e. species with site-specific populations, each of which is a resource specialist) (Dennis *et al.*, 2011; Nylin & Janz, 2009).

One aspect that probably influences plant-insect interactions is trophic level. Herbivores feed at the base of the food chain, on live, often photosynthesizing plant tissue. Drosophilids, on the other hand, consume yeasts and bacteria associated to decomposing plant tissue (Starmer & Fogleman, 1986) that has fallen or been dispersed. Yeasts and bacteria provide drosophilids with a source of nutrition and detoxification of harmful chemicals produced during decay of the host tissue, whilst drosophilids potentially contribute to microbiota dispersal and outbreeding (Gonzales, 2014): yeast tetraspores can survive in the drosophilid digestive tract and may be broken apart, enhancing random crossing among spores (Coluccio *et al.*, 2008). Consequently, contrary to herbivores, fruit-breeding drosophilids are not in an arms-race competition with plants but have mutualist interactions with their microbiome.

Final Remarks

The amount of research on drosophilid flies in the Neotropics has risen over the last few decades, but their breeding sites are still poorly known. The 100 nominal species of drosophilids recorded here represent about 10% of the known species of the Neotropics (Brake & Bachli,

2008). Among the remaining 90% of species, some breed in other substrata such as flowers or fungi (Gottschalk *et al.*, 2009), but most are unknown as to breeding sites. Future studies should provide new breeding records, and we strongly recommend that researchers provide a detailed description of breeding sites, including collection dates, geographic coordinates, habitat, and the conditions under which the material was kept in the laboratory. Ideally, substrate fragments (fruits, flowers, fungi) should be kept in separate containers in the laboratory, thus allowing compilation of negative results, i.e., empty resources, average abundance of flies emergent from each resource, and derived statistical analysis of resource occupancy. Additionally, efforts to identify both flies and hosts to species level should be made and vouchers of both deposited in collections. A compilation of this information can support tests of hypotheses such as the coexistence of competitors (MacArthur & Levins, 1964; Sevenster & Van Alphen, 1996) or niche breadth as a driver of geographical range size (Slatyer *et al.*, 2013).

Plant orders and families that are putatively non-hosting in the Neotropics, and that are fleshy-fruited, particularly common ones such as Piperales, Boraginales and Dipsacales should be targeted for *ad hoc* experiments to test if absence is stochastic or has an underlying cause. After such tests have been done, and with an improved spread of samples (some plant orders are very poorly sampled), it might be possible to discover if putative absences, as well as the very high diversity of drosophilids observed in certain orders (Arecales, Myrtales, and Solanales) is a sampling artefact or if it has an ecological or phylogenetic basis.

The apparent dearth of specialist drosophilids in the Neotropics is intriguing and might be an artefact of sampling bias or of an incomplete database. Alternatively, the recorded drosophilid generalism in the Neotropics could be real or an illusion. Drosophilids could, for example, be highly selective of the yeasts and bacteria colonizing host fruits, but if these bacteria and yeasts are not host specific, then crossing Drosophilids with host plant families will seem like generalism. The question if drosophilid generalism in the Neotropics is real, or

specialization masking as generalism, will only be clarified by the inclusion of additional data on the microbiota that are effectively feeding the larvae, i.e., by creating a tri-trophic model (plant/micro-organism/drosophilid). The interaction between drosophilids and their host organisms in the biologically diverse Neotropics seems a challenging but promising line of research to study the evolution of generalism and specialization.

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For Review Only

Supporting Information

SI 1 – Associations recorded between nominal drosophilids and nominal plant hosts. E = Exotic (red); N = Neotropical (green). Numbers within cells represent how many times (literature citations) the interaction was recorded. Interaction classes: E fly x E plant (upper left-hand quadrant – pink cells); E fly x N plant (upper right-hand quadrant – yellow cells); N fly x E plant (lower left-hand quadrant – blue cells); N fly x N plant (lower right-hand quadrant – green cells). Code = E or N followed by the species rank per interaction class. *D.* = *Drosophila*; *H.* = *Hirtodrosophila*; *N.* = *Neotanygastrella*; *S.* = *Scaptodrosophila*; *Z.* = *Zaprionus*.

SI 2 - *Zaprionus indianus* chasing away Neotropical *Drosophila nebulosa* from a fallen mango (*Mangifera indica* L.). Video recorded in an urban park (Parque Olhos D'Agua) in Brasília, Distrito Federal, Brazil, by R. Tidon on November 27th, 2017.

Contribution of authors: H. Valadão contributed to the design of the project, collected data from the literature and in the field, identified and incorporated fly vouchers, performed statistical analyses, prepared most figures and wrote the first draft of the paper. C. Proença contributed to the design of the project, identified plant hosts, incorporated plant vouchers into the herbarium, reviewed plant names and significantly contributed to the discussion in the final draft. M. Kuhlmann identified plant hosts, incorporated plant vouchers to the herbarium, reviewed plant names and prepared Figures 1 and 3. S. Harris contributed several important points to the discussion and performed the dispersion analysis. R. Tidon contributed to the design of the project, fly identification, statistical analyses and writing of the manuscript.

Table 1. Genera, species groups and species of Drosophilidae recorded in fruits in the Neotropical Region. The column “Plant O/F/S” refers to the total of plant Orders, Families and Species used by drosophilid taxon. E = Exotic; N = Neotropical. Numbers after N or E represent the drosophilid species rank in numbers of used hosts (see matrix in Appendix 1); no number indicates that either drosophilid or host species were not identified to species. Species authorship can be found in Bächli (2018). ^G generalist species. Greek letter Phi ϕ = included in a phylogeny. **R** (Russo et al., 2013); **Y** (Yassin, 2013).

Genus	Group	Species	Plant O/F/S	References [§]
<i>Chymomyza</i> ϕ RY	<i>procnemis</i> ϕ RY	<i>Chymomyza pectinifemur</i> (N34)	3/3/3	18
<i>Diathoneura</i> ϕ Y	ungrouped	<i>Diathoneura dubia</i> (N76)	1/1/1	20
		<i>D. opaca</i> (N)	1/1/1	20
		<i>D. taeniatipennis</i> (N77)	1/1/1	23
		<i>Diathoneura</i> sp.	1/1/1	18
<i>Drosophila</i> ϕ RY	<i>annulimana</i> ϕ RY	<i>Drosophila annulimana</i> (N59)	1/1/1	18
		<i>D. arauna</i> (N43)	2/2/2	29, 39
		<i>D. gibberosa</i> (N65) ϕ R	1/1/1	20
	<i>bromeliae</i> ϕ Y	<i>D. bromelioides</i> (N35)	4/4/6	18

<i>busckii</i> ♂ RY	<i>D. busckii</i> ^G (E8) ♂ RY	8/9/13	2, 11, 18, 21, 34, 42, 43
<i>calloptera</i>	<i>D. atrata</i> ^G (N19)	10/13/15	4, 35, 43
	<i>D. calloptera</i> (N62)	1/1/1	35
	<i>D. quadrum</i> (N53)	1/1/2	18, 35
	<i>D. shildi</i> (N73)	1/1/1	35
	Unidentified (<i>D. calloptera</i> group)	2/2/2	12
<i>canalineae</i> ♂ RY	<i>D. canalinea</i> (N36) ♂ RY	4/5/6	12, 22
<i>caponei</i>	<i>D. caponei</i> (N63)	1/1/1	40
<i>cardini</i> ♂ RY	<i>D. cardini</i> ^G (N9) ♂ RY	11/12/14	6, 14, 18, 21, 28, 29, 34, 43
	<i>D. cardinoides</i> ^G (N4)	13/22/33	6, 9, 11, 18, 26, 27, 29, 30, 34, 37, 43
	<i>D. neocardini</i> (N17)	6/8/8	11, 18
	<i>D. neomorpha</i> (N31) ♂ R	6/6/6	26, 32
	<i>D. polymorpha</i> ^G (N3) ♂ R	19/25/41	2, 4, 9, 11, 12, 16, 18, 21, 27, 28, 29, 30, 37, 39, 40, 43
	<i>D. polymorpha</i> aff.	1/2/2	18
	Unidentified (<i>D. cardini</i> group)	9/9/12	5, 20

<i>coffeata</i> ♂ Y	<i>D. coffeata</i> (N45)	1/1/1	22, 23
	<i>D. fuscolineata</i> (N64)	1/1/1	12
	<i>D. fuscolineata</i> aff.	1/1/1	20
<i>dreyfusi</i> ♂ RY	<i>D. briegei</i> (N)	1/1/1	18
	<i>D. camargoi</i> ^G (N23) ♂ RY	6/6/7	12, 22, 23, 32, 35
	<i>D. dreyfusi</i> (N46)	2/2/3	18, 35
	Unidentified (<i>D. dreyfusi</i> group)	1/1/1	22
<i>funnebris</i> ♂ RY	<i>D. funnebris</i> (E) ♂ R	1/1/1	41
<i>guarani</i> ♂ RY	<i>D. griseolineata</i> ^G (N10)	12/17/26	9, 11, 18, 24, 30, 39
	<i>D. guaraja</i> (N66)	1/1/1	40
	<i>D. guaru</i> (N) ♂ RY	1/1/2	18
	<i>D. maculifrons</i> ^G (N6) ♂ R	17/20/25	2, 9, 12, 18, 21, 30, 35, 37, 39, 40
	<i>D. ornatifrons</i> ^G (N14) ♂ R	11/13/14	2, 4, 18, 21, 29, 35, 37
<i>immigrans</i> ♂ RY	<i>D. immigrans</i> ^G (E5) ♂ RY	13/16/30	1, 2, 3, 8, 9, 14, 16, 18, 29, 30, 34, 35, 37, 40, 43
<i>melanogaster</i> ♂ RY	<i>D. ananassae</i> ^G (E7) ♂ R	10/15/25	6, 9, 18, 20, 26, 32, 34

	<i>D. kikkawai</i> ^G (E6) ♂ R	11/15/20	6, 11, 18, 39, 43
	<i>D. malerkotliana</i> ^G (E3) ♂ R	11/20/35	5, 6, 11, 18, 22, 23, 28, 32, 34, 35, 43
	<i>D. melanogaster</i> ^G (E4) ♂ RY	14/21/42	1, 3, 8, 9, 16, 18, 20, 26, 32, 34
	<i>D. simulans</i> ^G (E1) ♂ R	19/32/82	2, 3, 4, 5, 8, 9, 11, 12, 14, 16, 18, 20, 21, 22, 23, 28, 29, 30, 34, 35, 37, 40, 43
	Unidentified (<i>D. melanogaster</i> group)	5/6/7	16
<i>obscura</i> ♂ RY	<i>D. subobscura</i> (N38) ♂ R	1/2/4	1, 8
<i>pallidipennis</i> ♂ RY	<i>D. pallidipennis</i> ^G (N29) ♂ RY	6/6/7	9, 11, 18
<i>peruviana</i> ♂ Y	<i>D. peruviana</i> (N69) ♂ Y	1/1/1	12
<i>repleta</i> ♂ RY	<i>D. buzzatii</i> (N61) ♂ R	1/1/1	3
	<i>D. fulvimacula</i> ^G (N30)	4/4/4	20, 22, 23
	<i>D. hydei</i> ^{†G} (E10) ♂ RY	6/6/9	1, 3, 9, 16, 18, 21, 34, 43
	<i>D. mercatorum</i> ^G (N5) ♂ RY	7/19/29	2, 9, 16, 18, 21, 25, 29, 30, 34, 37, 40, 43
	<i>D. meridionalis</i> (N49)	2/2/2	16, 25

	<i>D. onca</i> ^G (N32)	4/4/5	11, 18, 40
	<i>D. paranaensis</i> (N37)	4/4/5	20, 40
	<i>D. peninsularis</i> (N)	1/1/1	25
	<i>D. ramsdeni</i> (N)	1/1/1	33
	<i>D. repleta</i> ^G (N33) ϕ R	6/6/6	4, 18, 20
	<i>D. senei</i> (N71)	1/1/1	18
	<i>D. zottii</i> ^G (N21)	5/7/8	18, 30, 37
	<i>D. onca</i> <i>aff.</i>	1/1/1	20
	<i>D. paranaensis</i> <i>aff.</i>	1/1/1	20
	<i>D. zottii</i> <i>aff.</i>	1/1/1	18
	Unidentified (<i>D. repleta</i> group)	6/6/7	12, 30, 37
<i>saltans</i> ϕ RY	<i>D. prosaltans</i> ^G (N25) ϕ R	4/5/6	9, 18, 35, 43
	<i>D. saltans</i> (N70) ϕ RY	2/2/2	18, 33
	<i>D. sturtevantii</i> ^G (N11) ϕ RY	10/14/17	11, 12, 18, 20, 21, 22, 23, 26, 29, 32, 34, 35, 43
	Unidentified (<i>D. saltans</i> group)	3/3/3	2, 22, 37

<i>tripunctata</i> ♂ RY	<i>D. albicans</i> (N57)	1/1/2	20
	<i>D. albirostris</i> (N58) ♂ R	1/1/2	32
	<i>D. angustibucca</i> ^G (N22)	6/7/8	2, 9, 26, 30
	<i>D. bandeirantorum</i> ^G (N16)	7/8/11	2, 9, 12, 18, 30, 37
	<i>D. bodemannae</i> (N60)	1/1/1	18
	<i>D. cuaso</i> ^G (N39)	3/3/3	6, 35, 39
	<i>D. frotapessoai</i> (N48)	1/1/2	18
	<i>D. medioimpressa</i> (N)	1/1/1	18
	<i>D. medioparva</i> (N41)	4/4/4	19, 26
	<i>D. mediopicta</i> ^G (N28)	3/3/4	18, 30, 37
	<i>D. mediopictoides</i> (N) ♂ R	1/1/1	18
	<i>D. mediopunctata</i> ^G (N12)	12/16/22	11, 18, 30, 34, 43
	<i>D. mediosignata</i> ^G (N24)	8/10/11	18, 23, 24, 35
	<i>D. mediotriata</i> ^G (N8) ♂ R	12/14/23	6, 11, 12, 18, 20, 21, 28, 29, 34, 35, 43
	<i>D. mesostigma</i> (N50)	2/2/2	22, 29
	<i>D. metzii</i> (N67) ♂ R	1/1/1	32

	<i>D. nappae</i> (N51)	2/2/2	18
	<i>D. neoguaramunu</i> (N)	3/4/4	18, 22
	<i>D. paraguayensis</i> ^G (N7)	15/25/29	11, 18, 23, 26, 29, 30, 32, 39, 43
	<i>D. paramediotriata</i> ^G (N15)	7/10/10	18, 32, 34, 35, 43
	<i>D. pellewae</i> (N52)	1/1/1	18
	<i>D. setula</i> (N54)	1/1/1	18
	<i>D. trapeza</i> ^G (N26)	4/4/5	19, 20, 34
	<i>D. trifilum</i> (N74)	1/1/1	39
	<i>D. tripunctata</i> (N75) ϕ RY	1/1/1	9
	<i>D. tristriata</i> (N)	1/1/2	18
	<i>D. metzii</i> aff.	2/2/3	20
	<i>D. paraguayensis</i> aff.	2/2/2	20
	Unidentified (<i>D. tripunctata</i> group)	13/16/21	2, 12, 24, 37
<i>virilis</i> ϕ RY	<i>D. virilis</i> (E11) ϕ RY	1/1/1	18
<i>willistoni</i> ϕ RY	<i>D. bocainensis</i> ^G (N27)	6/7/8	18, 29, 35, 43
	<i>D. bocainoides</i> (N44)	3/3/3	18

		<i>D. capricorni</i> ^G (N20) φ R	8/10/14	18, 35, 39, 43
		<i>D. equinoxialis</i> (N47) φ R	2/2/2	23, 32
		<i>D. fumipennis</i> ^G (N13) φ R	6/10/15	11, 12, 18, 20, 22, 23, 28, 35, 37, 43
		<i>D. nebulosa</i> ^G (N1) φ RY	19/28/57	4, 5, 6, 7, 9, 11, 12, 14, 16, 18, 20, 21, 22, 23, 26, 28, 29, 30, 32, 34, 35, 37, 39, 43
		<i>D. parabocainensis</i> (N68)	1/1/1	39
		<i>D. paulistorum</i> ^G (N18) φ R	7/8/10	23, 31, 32, 39, 43
		<i>D. sucinea</i> (N55) φ R	3/3/3	20
		<i>D. tropicalis</i> (N56) φ R	2/2/2	23, 32
		<i>D. willistoni</i> ^G (N2) φ RY	21/28/41	2, 4, 9, 12, 14, 16, 23, 28, 29, 30, 31, 32, 35, 36, 37, 38, 39, 40, 42
		Unidentified (<i>D. willistoni</i> subgroup)	18/30/62	5, 6, 11, 12, 18, 20, 22, 24, 34, 43
	Ungrouped	<i>D. aracea</i> (N) φ R	1/1/2	20
		<i>D. serenensis</i> (N72)	1/1/1	10
<i>Hirtodrosophila</i> φ RY	Ungrouped	<i>H. pictiventris</i> (N78) φ RY	1/1/1	20

<i>Mycodrosophila</i> ♂ RY	Ungrouped	<i>Mycodrosophila</i> sp.	1/1/1	35
<i>Neotanygastrella</i> ♂ Y	Ungrouped	<i>N. tricoloripes</i> (N40) ♂ Y	3/3/3	15, 23, 35
<i>Rhinoleucophenga</i> ♂ Y	Ungrouped	<i>R. bivisualis</i> (N42) ♂ Y	2/2/2	20
		<i>Rhinoleucophenga</i> sp.	7/10/11	11, 18, 29, 34, 43
<i>Scaptodrosophila</i> ♂ RY	<i>latifasciaeformis</i> ♂ RY	<i>S. latifasciaeformis</i> ^G (E9) ♂ RY	8/9/9	18, 21, 23, 29, 34, 43
<i>Scaptomyza</i> ♂ RY		<i>Scaptomyza</i> sp.	1/1/1	18
<i>Zaprionus</i> ♂ RY	<i>vittiger</i> ♂ RY	<i>Z. indianus</i> ^G (E2) ♂ RY	16/25/41	6, 11, 13, 14, 17, 18, 21, 29, 34, 35, 39, 43
<i>Zygothrica</i> ♂ Y		<i>Zygothrica</i> sp.	5/5/6	11, 18

1
2 §References. 1: (Alamiri, 2000), 2: (Araújo & Valente, 1981), 3: (Beltrami *et al.*, 2010), 4: (Birch & Battaglia, 1957), 5: (Bizzo & Sene, 1982), 6:
3 (Blauth & Gottschalk, 2007), 7: (Bonorino & Valente, 1989), 8: (Brncic & Budnik, 1987), 9: (Brncic & Valente, 1978), 10: (Brncic, 1957), 11:
4 (De Toni *et al.*, 2001), 12: (Dobzhansky & Pavan, 1950), 13: (Fernandes & Araújo, 2011), 14: (Fernandes *et al.*, 2009), 15: (Frota-Pessoa &
5 Wheeler, 1951), 16: (Goñi *et al.*, 1998), 17: (Goñi *et al.*, 2001), 18: (Gottschalk, 2008), 19: (Heed & Wheeler, 1957), 20: (Heed, 1957), 21: (Leão
6 & Tidon, 2004), 22: (Martins & Santos, 2007), 23: (Martins, 1996), 24: (Pavan, 1959), 25: (Pereira *et al.*, 1983), 26: (Pipkin, 1965), 27: (Rohde &
7 Valente, 1996), 28: (Roque & Tidon, 2008), 29: (Roque *et al.*, 2009), 30: (Saavedra *et al.*, 1995), 31: (Santos & Valente, 1990), 32: (Sevenster &
8 Van Alphen, 1996), 33: (Sturtevant, 1917), 34: (Valadão & Tidon, 2015), 35: (Valadão *et al.*, 2010), 36: (Valente & Araujo, 1986), 37: (Valente
9 & Araújo, 1991), 38: (Valente *et al.*, 2003), 39: (Vilela & Selivon, 2000), 40: (Vilela, 2001), 41: (Wheeler *et al.* 1962), 42: (Wyckhuys *et al.*, 2012),
10 43: New records from the Laboratório de Biologia Evolutiva, Universidade de Brasília, Brazil.

Table 2. Plant families and species whose fruits were used as breeding sites for drosophilids in the Neotropics. ? = geographical provenance uncertain; E = Exotic; N = Neotropical. Numbers after N or E represent the host species rank in numbers of hosted drosophilids (see matrix in Appendix 1); no number indicates that either host plant or drosophilid were not identified to species. New host records at family, genus and species level in the Neotropics in **bold**.

Family	Species
Anacardiaceae	<i>Anacardium giganteum</i> Hancock ex Engl. (N61)
	<i>Anacardium humile</i> A.St.-Hil. (N62)
	<i>Mangifera indica</i> L. (E11)
	<i>Spondias mombin</i> L. (N10)
Annonaceae	<i>Annona muricata</i> L. (N73)
	<i>Annona</i> sp. (?)
	<i>Xylopia</i> sp. (?)
Apocynaceae	<i>Hancornia speciosa</i> Gomes (N48)
	<i>Parahancornia fasciculata</i> (Poir.) Benoist (N6)
Aquifoliaceae	<i>Ilex ovalifolia</i> Bonpl. ex Miers (N49)
Araceae	<i>Anthurium</i> sp. (N)
	<i>Philodendron bipinnatifidum</i> Schott ex Endl. (N19)
	<i>Syngonium</i> sp.1 (N)
	<i>Syngonium</i> sp.2 (N)
	<i>Xanthosoma</i> sp. (N)
Araliaceae	<i>Dendropanax arboreus</i> (L.) Decne. & Planch. (N74)
	<i>Schefflera morototoni</i> (Aubl.) Maguire, Steyerm. & Frodin. (N33)
	<i>Schefflera</i> sp. (?)

Arecaceae	<i>Acrocomia aculeata</i> (Jacq.) Lodd. ex Mart. (N39)
	<i>Astrocaryum murumuru</i> Mart. (N40)
	<i>Bactris gasipaes</i> Kunth. (N75)
	<i>Bactris</i> sp. (N)
	<i>Butia capitata</i> (Mart.) Becc. (N28)
	<i>Butia eriospatha</i> (Mart. ex Drude) Becc. (N34)
	<i>Butia yatay</i> (Mart.) Becc. (N82)
	<i>Cocos nucifera</i> L. (N63)
	<i>Cryosophila warscewiczii</i> (H.Wendl.) Bartlett (N76)
	<i>Mauritia flexuosa</i> L.f. (N3)
	<i>Oenocarpus distichus</i> Mart. (N77)
	<i>Syagrus romanzoffiana</i> (Cham.) Glassman (N1)
	<i>Syagrus</i> sp. (N)
Balanophoraceae	<i>Helosis</i> sp. (N)
Bromeliaceae	<i>Ananas</i> sp. (N)
	<i>Bromelia antiacantha</i> Bertol. (N50)
	<i>Bromelia balansae</i> Mez (N41)
Cactaceae	<i>Cereus fernambucensis</i> Lem. (N42)
	<i>Opuntia ficus-indica</i> (L.) Mill. (N64)
Caricaceae	<i>Carica papaya</i> L. (N83)
	<i>Jacaratia</i> sp. (N)
Caryocaraceae	<i>Caryocar brasiliense</i> A.St.-Hil. (N5)
Celastraceae	<i>Cheiloclinium cognatum</i> (Miers) A.C.Sm. (N81)
	<i>Salacia multiflora</i> (Lam.) DC. (N52)
Clusiaceae	<i>Clusia grandiflora</i> Splitg. (N78)

	<i>Clusia</i> sp. (N)
	<i>Garcinia gardneriana</i> (Planch. & Triana) Zappi (N65)
Combretaceae	<i>Buchenavia tomentosa</i> Eichler (N25)
	<i>Terminalia catappa</i> L. (E3)
Cucurbitaceae	<i>Cucurbita</i> sp. (N)
Cyclanthaceae	<i>Carludovica palmata</i> Ruiz & Pav.(N95)
Ebenaceae	<i>Diospyros hispida</i> A.DC. (N53)
	<i>Diospyros inconstans</i> Jacq. (N16)
	<i>Diospyros kaki</i> Thunb. (E8)
	<i>Diospyros lotus</i> L. (E23)
Ericaceae	<i>Arbutus unedo</i> L. (E25)
Euphorbiaceae	<i>Aleurites moluccana</i> (L.) Willd. (E5)
	<i>Euphorbia phosphorea</i> Mart. (N43)
	<i>Hura crepitans</i> L. (N66)
Fabaceae	<i>Andira humilis</i> Mart. ex Benth. (N67)
	<i>Andira inermis</i> (Wright) DC. (N29)
	<i>Enterolobium cyclocarpum</i> (Jacq.) Griseb. (N84)
	<i>Holocalyx balansae</i> Micheli (N20)
	<i>Hymenaea martiana</i> Hayne (N85)
	<i>Inga sapindoides</i> Willd. (N35)
	<i>Inga vera</i> (Willd.) J.Leon (N68)
	<i>Inga</i> sp. (N)
	<i>Phaseolus vulgaris</i> L. (N21)
Flacourtiaceae	<i>Dovyalis caffra</i> (Hook. f. & Harv.) Warb. (E16)
Ginkgoaceae	<i>Ginkgo biloba</i> L. (E12)

Lauraceae	<i>Cinamomum</i> sp. (?)
	<i>Nectandra grandiflora</i> Nees & Mart. (N69)
	<i>Ocotea</i> sp. (?)
	<i>Persea americana</i> Mill. (N86)
Liliaceae	<i>Dracaena fragrans</i> (L.) Ker Gawl. (E26)
Loganiaceae	<i>Strychnos brasiliensis</i> (Spreng.) Mart. (N54)
	<i>Strychnos pseudoquina</i> A. St.-Hil. (N14)
Magnoliaceae	<i>Magnolia</i> sp. sect. <i>Talauma</i> (N)
Malpighiaceae	<i>Byrsonima basiloba</i> A.Juss. (N70)
	<i>Byrsonima ligustrifolia</i> Mart. (N44)
	<i>Byrsonima verbascifolia</i> (L.) Rich. ex Juss. (N55)
	<i>Byrsonima</i> sp. (N)
	Malpighiaceae (N)
Malvaceae	<i>Chorisia</i> sp. (?)
	<i>Matisia cordata</i> Bonpl. (N87)
	<i>Pseudobombax</i> sp. (N)
Meliaceae	<i>Cabrlea canjerana</i> (Vell.) Mart.(N45)
	<i>Cabrlea</i> sp. (N)
	<i>Guarea</i> sp. (N)
	<i>Melia azedarach</i> L.(E7)
Metteniusaceae	<i>Emmotum nitens</i> (Benth.) Miers (N8)
Moraceae	<i>Artocarpus altilis</i> (Parkinson ex F.A.Zorn) Fosberg (E17)
	<i>Artocarpus heterophyllus</i> Lam. (E 33)
	<i>Brosimum alicastrum</i> Sw. (N56)
	<i>Brosimum gaudichaudii</i> Trécul (N30)

	<i>Castilla elastica</i> Cerv. (N26)
	<i>Coussapoa</i> sp.(N)
	<i>Ficus carica</i> L. (E9)
	<i>Ficus cestrifolia</i> Schott. (N17)
	<i>Ficus</i> sp. (?)
	<i>Helicostylis</i> sp. (N)
	<i>Maclura pomifera</i> (Raf.) C.K.Schneid. (N88)
	<i>Maclura tinctoria</i> (L.) D.Don ex Steud. (N9)
	<i>Maclura</i> sp. (?)
	<i>Morus alba</i> L. (E21)
	<i>Morus nigra</i> L. (E27)
Musaceae	<i>Musa</i> sp. (E)
Myrtaceae	<i>Acca sellowiana</i> (O.Berg) Burret. (N89)
	<i>Campomanesia guazumifolia</i> (Cambess.) O.Berg (N46)
	<i>Campomanesia phaea</i> (O.Berg) Landrum (N57)
	<i>Campomanesia pubescens</i> (Mart. ex DC.) O.Berg (N90)
	<i>Campomanesia xanthocarpa</i> (Mart.) O.Berg (N22)
	<i>Campomanesia</i> sp.(N)
	<i>Eugenia brasiliensis</i> Lam. (N36)
	<i>Eugenia dysenterica</i> DC. (N71)
	<i>Eugenia uniflora</i> L. (N23)
	<i>Eugenia</i> sp. (?)
	<i>Myrcia</i> sp.(N)
	<i>Myrcia spectabilis</i> DC. (N31)
	<i>Psidium cattleianum</i> Afzel. ex Sabine (N27)

	<i>Psidium guajava</i> L. (N2)
	<i>Psidium salutare</i> (O.Berg) Landrum (N79)
	<i>Psidium</i> sp. (N)
	<i>Syzygium cumini</i> (L.) Skeels (E13)
	<i>Syzygium jambos</i> (L.) Alston (E22)
	<i>Syzygium</i> sp. (E)
Olacaceae	<i>Heisteria</i> sp. (?)
Oxalidaceae	<i>Averrhoa carambola</i> L. (E4)
Passifloraceae	<i>Passiflora edulis</i> Sims (N7)
	<i>Passiflora ligularis</i> Juss. (N80)
	<i>Passiflora quindensis</i> Killip (N47)
	<i>Passiflora</i> sp. (?)
Phytolaccaceae	<i>Phytolacca dioica</i> L. (N58)
Quiinaceae	<i>Lacunaria jenmanii</i> (Oliv.) Ducke (N)†
Rhamnaceae	<i>Hovenia dulcis</i> Thunb. (E28)
	<i>Ziziphus joazeiro</i> Mart. (N91)
Rosaceae	<i>Eriobotrya japonica</i> (Thunb.) Lindl. (E2)
	<i>Fragaria</i> sp. (E)
	<i>Malus domestica</i> Borkh. (E29)
	<i>Malus sylvestris</i> Mill. (E18)
	<i>Malus</i> sp. (E)
	<i>Prunus armeniaca</i> L. (E30)
	<i>Prunus persica</i> (L.) Stokes (E14)
	<i>Prunus subcoriacea</i> (Chodat & Hassl.) Koehne (N94)
	<i>Prunus</i> sp. (?)

	<i>Pyrus</i> sp. (E)
	<i>Rubus idaeus</i> L. (E19)
	<i>Rubus urticifolius</i> Poir. (E31)
Rubiaceae	<i>Alibertia edulis</i> (Rich.) A.Rich. ex DC. (N32)
	<i>Coffea arabica</i> L. (E10)
	<i>Posoqueria latifolia</i> (Rudge) Schult. (N37)
	<i>Posoqueria</i> sp. (N)
	<i>Psychotria</i> sp. (?)
	<i>Randia armata</i> (Sw.) DC. (N12)
Rutaceae	<i>Citrus aurantium</i> L. (E32)
	<i>Citrus</i> × <i>bergamia</i> Risso & Poit. (E24)
	<i>Citrus limon</i> (L.) Burm. f. (E20)
	<i>Citrus reticulata</i> Blanco (E6)
	<i>Citrus sinensis</i> (L.) Osbeck (E1)
	<i>Citrus</i> sp. (E)
Sapindaceae	<i>Allophylus edulis</i> (A.St.-Hil., A.Juss. & Cambess.) Radlk (N92)
	<i>Litchi</i> sp. (E)
Sapotaceae	<i>Chrysophyllum cainito</i> L. (N93)
	<i>Chrysophyllum gonocarpum</i> (Mart. & Eichler ex Miq.) Engl. (N24)
	<i>Chrysophyllum</i> sp. (?)
	<i>Lucuma</i> sp. (?)
	<i>Manilkara zapota</i> (L.) P.Royen (N38)
	<i>Pouteria ramiflora</i> (Mart.) Radlk. (N11)
	<i>Pouteria viridis</i> (Pittier) Cronquist. (N96)
Siparunaceae	<i>Siparuna thecaphora</i> (Poepp. & Endl.) A.DC. (N72)

Solanaceae	<i>Capsicum annuum</i> L. (N18)
	<i>Solanum americanum</i> Mill. (N59)
	<i>Solanum lycocarpum</i> A. St.-Hil. (N4)
	<i>Solanum lycopersicum</i> Lam. (N13)
	<i>Solanum reitzii</i> L.B. Sm. & Downs (N60)
Thymelaeaceae	<i>Daphnopsis</i> sp. (N)
Urticaceae	<i>Cecropia glaziovii</i> Snethl. (N51)
	<i>Cecropia</i> sp. (N)
Verbenaceae	<i>Citharexylum donnell-smithii</i> Greenm. (N97)
	<i>Citharexylum myrianthum</i> Cham. (N15)
Vitaceae	<i>Vitis vinifera</i> L. (E15)

Table 3. Percentages of observed interactions (matrix cell occupancy) between drosophilids and host species (i.e. 100% would be all possible drosophilids using all possible hosts) for each of four possible classes of interaction: 1) neotropical drosophilid x neotropical host; 2) neotropical drosophilid x exotic host; 3) exotic drosophilid x neotropical host; and 4) exotic drosophilid x exotic host. We used the value of 6.6% as the random expected occupancy in each category. The original matrix is available as Supporting Information.

Drosophilids (89 species)	Plant Hosts Fruits (130 species)	
	Neotropical	Exotic
Neotropical	$\frac{411}{7566} = 5.4\% \text{ **}$	$\frac{100}{2574} = 3.9\% \text{ **}$
Exotic	$\frac{169}{1067} = 15.8\% \text{ **}$	$\frac{85}{363} = 23.4\% \text{ **}$

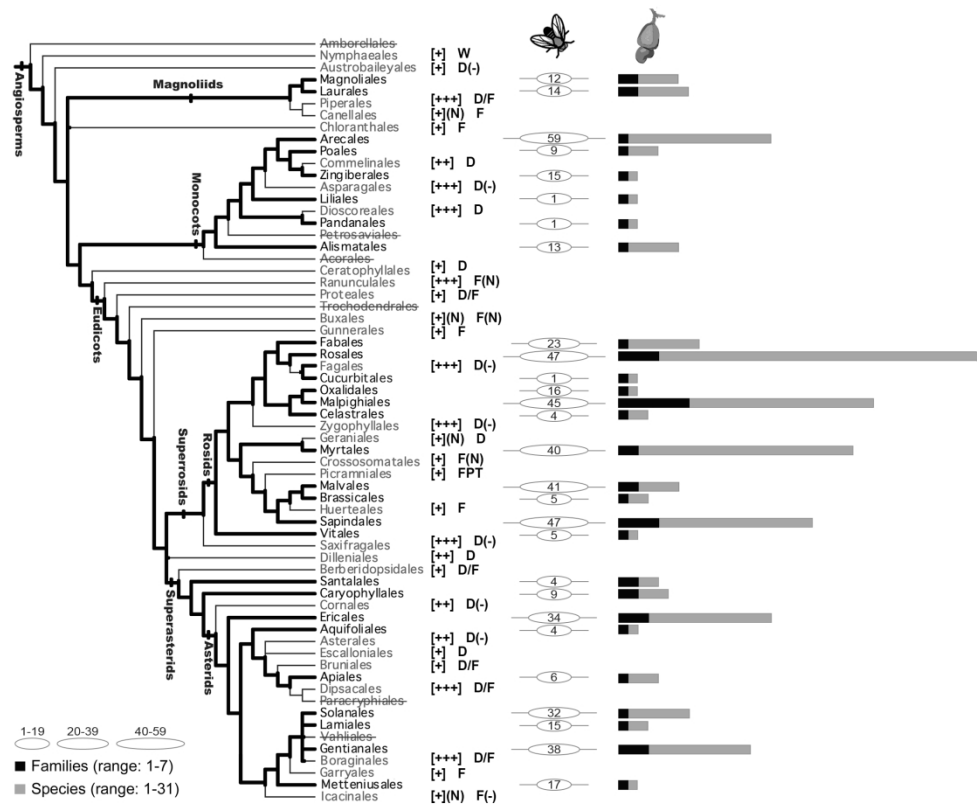


Fig. 1 Records of fruit-breeding drosophilid species plotted on the Angiosperm phylogeny at order level. Angiosperm phylogeny and plant orders based on APG IV, 2016). Numbers in winged balloons are numbers of recorded drosophilid species per order. Bold = hosting Neotropical Orders; Plain text = non-hosting Neotropical orders; Strikethrough = non-Neotropical orders; black bars = recorded host families; grey bars = recorded host species. Order richness: [+++] = >1,000 species; [++] = between 100-999 species; [+] <99 species. Fruit type: D = dry; D/F = dry or fleshy; FPT = fleshy with thin pericarp <0.5mm; W = water fruits that mature submerged in some aquatic plants. Qualifiers: (-) = with some exceptions; (N) = in the Neotropics. Fly image is a stylized *Drosophila melanogaster* (Meigen, 1830); fruit image is a stylized *Anacardium occidentale* L. fruit, the cashew.

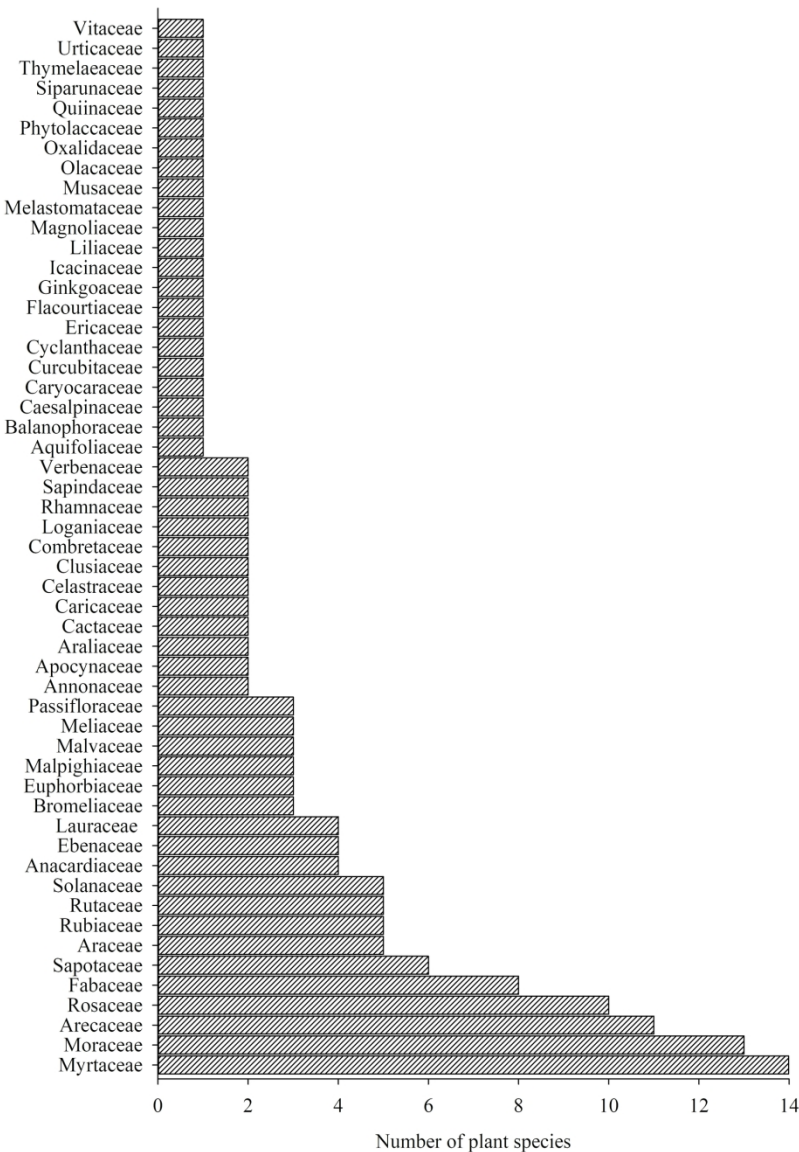


Fig. 2 Botanical families whose fruits were used as breeding sites for drosophilid larvae in the Neotropics ranked by numbers of host plant species.

159x226mm (300 x 300 DPI)

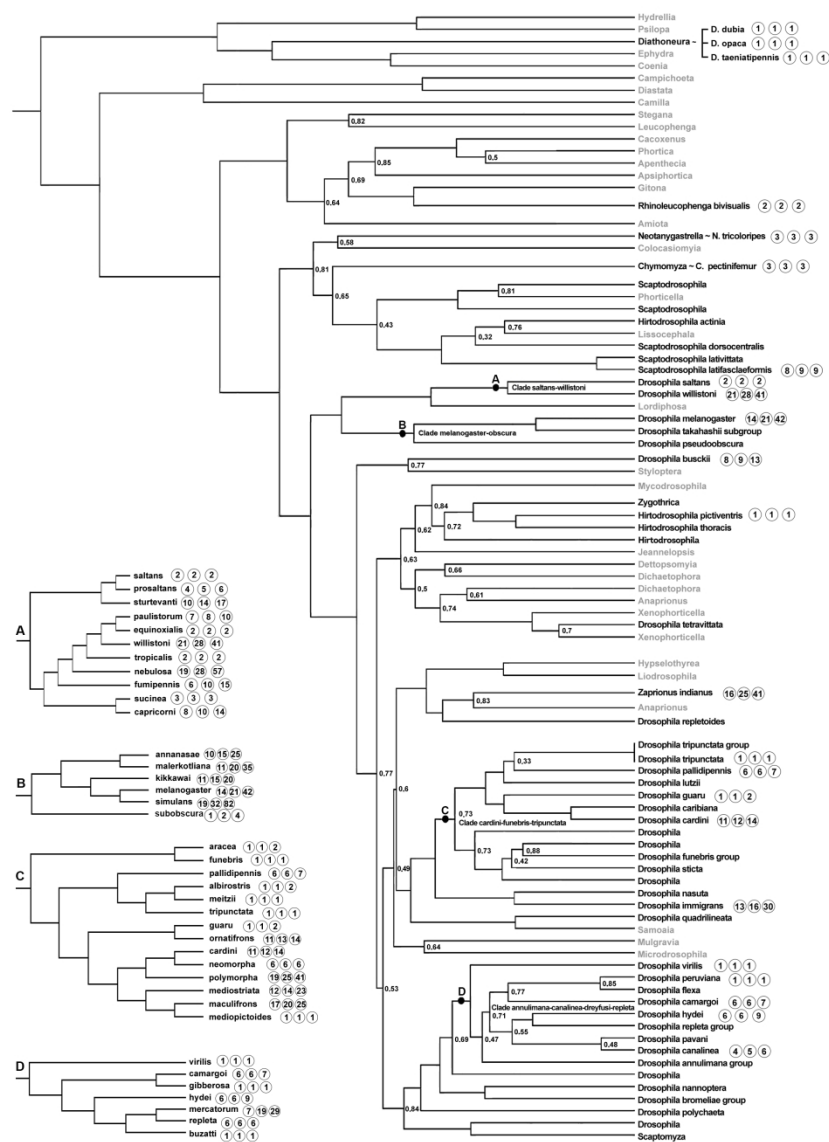


Fig. 3 Richness of host usage plotted on the Drosophilid phylogeny at genus and species level. Numbers within circles represent orders, families and nominal species of host plants. Right side: Phylogram constructed by Bayesian Inference adapted from Yassin (2013); most clades with no host plant data have been collapsed; only nodes with low posterior probabilities (PP < 0.9) show PP values; genera in pale grey are either non-hosting or not found in the Neotropics. Left side: Basic cladograms for four hosting clades of *Drosophila* reconstructed according to the phylogenetic hypothesis proposed by Russo et al. (2013): A) Clade *D. saltans* - *D. willistoni*; B) Clade *D. melanogaster* - *D. obscura*; C) Clade *D. cardini* - *D. funebris* - *D. tripunctata*; D) Clade *D. annulimana* - *D. canalinea* - *D. dreyfusi* - *D. repleta*. Phylogenetic hypothesis for the species present in our study were congruent between Yassin (2013) and Russo et al. (2013).

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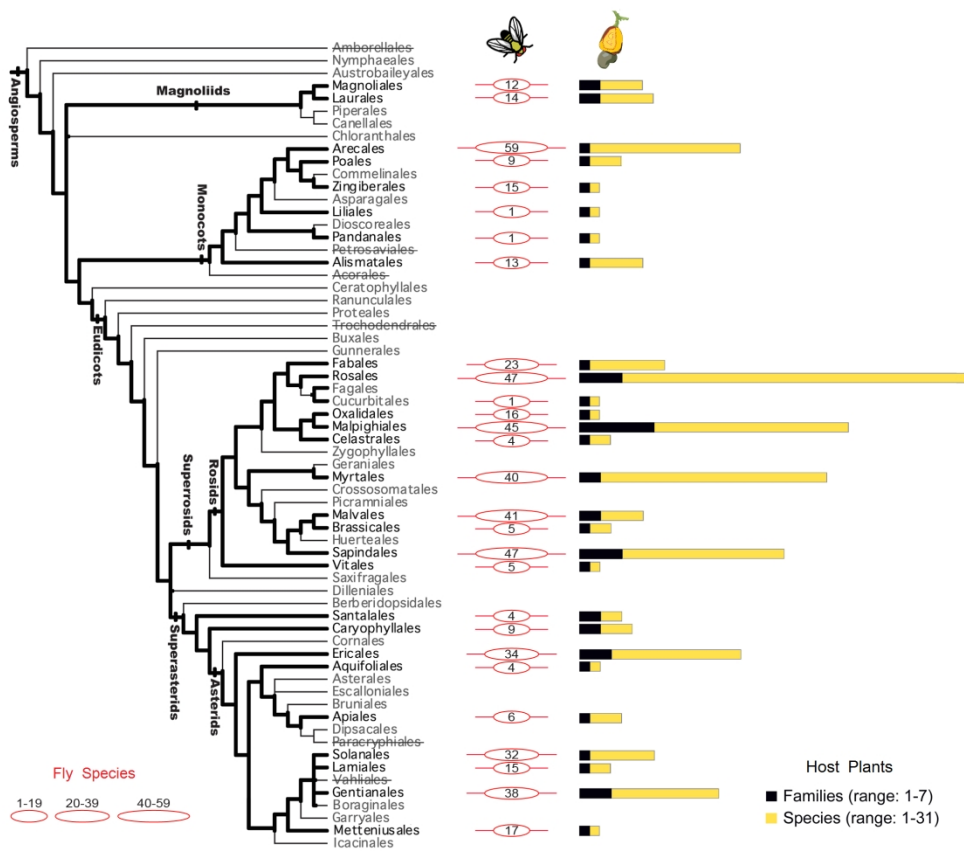
Legend for Supporting Information 1

SI 1 – Associations recorded between nominal drosophilids and nominal plant hosts. E = Exotic (red); N = Neotropical (green). Numbers within cells represent how many times (literature citations) the interaction was recorded. Interaction classes: E fly x E plant (upper left-hand quadrant – pink cells); E fly x N plant (upper right-hand quadrant – yellow cells); N fly x E plant (lower left-hand quadrant – blue cells); N fly x N plant (lower right-hand quadrant – green cells). Code = E or N followed by the species rank per interaction class. *D.* = *Drosophila*; *H.* = *Hirtodrosophila*; *N.* = *Neotanygastrella*; *S.* = *Scaptodrosophila*; *Z.* = *Zaprionus*.

We recorded 100 nominal fruit-breeding species of drosophilids; 49 out of 50 eligible ($N > 4$) species were fruit generalists; we argue that drosophilids could be selective of host tissue microbiota.

Plant species (180) recorded as drosophilid hosts are distributed across the main Angiosperm lineages and fleshy-fruited orders; plant families that hosted the most drosophilid species were Arecaceae, Moraceae, and Myrtaceae.

Drosophilids exotic to the Neotropics breed in more plant species than native Neotropical drosophilids possibly because they are generalists that have survived the trials of introduction into the Neotropics.



195x168mm (300 x 300 DPI)