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Exceptional Species Diversity of Drosophilidae (Diptera) in a Neotropical Forest

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ABSTRACT

The highest single-site species diversity known thus far in the world for Drosophilidae is in Costa Rica, based on findings in this report. A total of 352 species of Drosophilidae (Diptera) were found in a cloud forest (1580 m) in Zurquí de Moravia, San José Province (hereafter "Zurquí"), based on 2908 specimens collected continuously for one year, using eight trapping and collecting methods. There are currently 305 described species from Costa Rica. Zurquí is at the edge of a large, protected area and was the site of an All-Diptera inventory project. For this study, drosophilid specimens were identified to genus/subgenus, sorted to morphospecies, and their abundances plotted by collection method: Malaise traps, flight intercept traps, baited traps, light and yellow pan traps, emergence traps, and hand collecting with nets. The standard method used by drosophilists, bait trapping, captured a small fraction of species. Malaise traps captured 87% of all species, and 41% of the 352 species were captured only this way. Emergence traps captured a surprising diversity (47 species) of *Diathoneura* and *Drosophila*, establishing that leaf litter/humus is an important breeding site for some taxa. Combining all collection methods, 11 species were abundant, as defined by 50 or more specimens, and comprised 35% of all specimens in the study; two-thirds (66%) of all species were rare, as defined by five or fewer specimens. Comparisons are made to other well-collected sites and regions around the world. Lowland to mid-montane forests on the eastern slopes of the Andes may be the most diverse area for Drosophilidae, a family that is exemplary for studying the ecology and evolution of tropical diversity.

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INTRODUCTION

The intense focus on a few species of *Drosophila* for experimental work has propelled the fields of genetics and development, while also distracting biologists from an appreciation for the rich natural history of Drosophilidae. With ~4650 described species as of 2022 (Bächli, 2022), Drosophilidae is one of the larger families of Cyclorrhapha. It is also one of the most ecologically diverse families in Diptera, which makes *Drosophila* and the family excellent for comparative biology. There are species and whole groups that breed in flowers, even specialized pollinators of orchids (Endara et al., 2010; Policha et al., 2019), or that breed in myriad kinds of fungi and fruits, under decaying bark, and others that mine stems and leaves, (Brncic, 1983; Jones et al., 2021; Lachaise and Tsacas, 1983; Lacy, 1982; Pipkin et al., 1966; Policha et al., 2019; Shorrocks, 1977; Valer et al., 2016; Van Klinken and Walter, 2001a; Vaz et al., 2014; dos Santos and Vilela, 2005). Larvae of some species are predatory on other insects (Ashburner, 1981) or are parasites of arthropods (Grimaldi and Nguyen, 1999; Carvalho-Filho et al., 2018).

Taxonomic, life-history, and field work on Drosophilidae has hardly kept pace with experimental work. For example, biotic surveys in northern, temperate regions have well documented the drosophilids there (e.g., Bächli et al., 2004; Miller et al., 2017), but we don't really know how many species of them live, for example, within a typical tropical forest. Since they are mostly a tropical group, knowing such information would be fundamental to understanding basic aspects of their diversity, distribution, and biology.

The current paper expands upon and revises initial efforts on the Drosophilidae contributed to the ZADBI project (Zurquí All-Diptera Biodiversity Inventory) (Borkent et al., 2018; Brown et al., 2018). The project intensively sampled for all species of Diptera in a small section of mid-montane cloud forest in San José Province, Costa Rica, using most standard trapping and other collecting methods for insects. For those reports a total of 219 drosophilid species were reported, but only a portion of the drosophilid samples were then sorted and analyzed. That made Drosophilidae the sixth most diverse family at Zurquí at the time, behind (in order of most diverse first) Cecidomyidae, Phoridae, Tachinidae, Mycetophilidae, and Tipulidae. Now, with samples fully sorted to 352 species, Drosophilidae is the third most diverse family at Zurquí (there were 404 species of Phoridae, and 286 species of Tachinidae). Thus, there are more species of Drosophilidae at Zurquí than any other family of Schizophora flies (acalyptrates and calyptrates). As is documented here, that diversity is extremely uneven among genera and other groups of Drosophilidae as well as across collecting methods.

MATERIALS AND METHODS

The protocols for collecting and details about the site are provided in Borkent et al. (2018) and Brown et al. (2018), which are briefly summarized here.

Site: The site is a cloud forest 150 m × 226 m in Zurquí de Moravia, San José Province, Costa Rica, situated at 10.47N, 84.008W at approximately 1580 m altitude (fig. 1). The forest has a small ridge, a perennial and a seasonal stream, adjacent to some small pastures, with Braulio Carillo National Park to the north and agricultural land to the south (fig. 1). Braulio

FIG. 1. Satellite photos of the study site at Zurquí, Costa Rica (red bubble). **A.** Showing the site at the northern edge of agricultural development, and at the southern edge of protected montane forest. **B.** Closer view of study site. The peninsula of forest surrounded by pasture and some adjacent forest is the study site. Images courtesy of Google Maps©.

Carillo comprises 47,500 hectares (108,000 acres) of undisturbed forests (Talamancan cloud forest to lowland rainforest) as well as four main peaks (up to 3000 m elevation); it is clearly a rich source for the species in the Zurquí forest site.

Collection Methods: A combination of passive and active traps were used from September 2012 to October 2013. Passive traps (fig. 2) intercept flying or emerging specimens (e.g.,

FIG. 2. Three types of passive trapping used at the Zurquí study site. **A.** Malaise trap. **B.** Flight intercept trap. **C, D.** Emergence traps. Photos by Art Borkent.

Malaise/flight-intercept traps, emergence traps). Active traps exploit the behavior of flies to various attractants, including several wavelengths of light (light traps), various baits, and colors (yellow pan traps). While passive traps have inherently less sampling bias, they still bias toward those species with longer flight periods or that either have more active flight or fly and emerge near the ground. The only significant sampling method that was not used is canopy fogging. Overall, the effects of sampling bias in any one method are likely smoothed out by the combined use of various collection methods. The temporal consistency of sampling reduces sampling bias due to seasonal changes.

Collecting was also done for a week by the senior author, and at intermittent times by parataxonomists, using a hand net to target discrete sites where certain taxa can be found. This involved the use of short- and long-handled nets with very fine, soft netting, which were used by sweeping quickly and repeatedly several inches above various substrates: macrofungi, rotting logs, forest-floor leaf litter, over fallen flowers/fruit, and along the trunks and lower branches of trees. Yearlong sampling used two Townes-style Malaise traps (Sante Traps©) (fig. 2A) pitched on the ground in distant parts of the forest site; a third was suspended just below the canopy but was not productive. A flight-intercept trap was placed at the edge of the forest, using pans of preservative beneath the screening to capture the flies that fall and an overhead tarp to prevent rain from flooding the pans (fig. 2B). Three types of light traps were used: A bucket-type, CDC-type, and UV-pan. Yellow pan traps used small plastic bowls with liquid preservative. Emergence traps (fig. 2C, D) were placed in various areas of the forest, such as over leaf litter, branches, and a small stream. The traps were emptied regularly.

Bulk samples from the traps were processed by technicians and parataxonomists at the former INBio (Instituto Nacional da Biodiversidade), in Heredia, Costa Rica. This included separating the specimens from the bulk samples, drying them in the solvent HMDS (hexamethyldisilazane) (samples were initially preserved in ethanol), point-mounting, labelling, and adding barcode labels. Specimens were then barcode scanned at the AMNH, sorted into genera and species, and the IDs entered into an Excel spreadsheet. For the five most common species of *Drosophila* (in both major subgenera, *Drosophila* and *Sophophora*), only a portion were mounted. Thus, the abundance of these very common species is lower than indicated. The senior author examined some of the screened residues for duplicate specimens and can confirm that the INBio technicians were highly skilled and thorough in mounting specimens of all other species.

SORTING: Mounted and labelled specimens were sorted to species using external features: body size, body and wing pigmentation patterns, chaetotaxy of head and thoracic bristles, and various specialized features (aristal structure, carina, costal lappet, foreleg structure, oviscapt structure, sexual dimorphisms, etc.). Genera whose species' external color patterns make sorting easy include, for example, *Diathoneura* (fig. 4), *Stegana* (fig. 3B), and *Zygothrica* (fig. 9), among others. For other groups, where pigmentation and chaetotaxy differences are subtle, it was necessary to also dissect male and female genitalia, to verify the sorting based on external features (e.g., *Microdrosphila* [fig. 3], *Cladochaeta* [fig. 5], and various groups of *Drosophila* [fig. 6]).

All mounted *Drosophila* specimens were sorted into subgenera (*Drosophila*, *Sophophora*), and some of these into species groups. No specimens of Drosophilidae in the study were identified to named species, for several reasons: (1) This would take substantially more time; (2) there is a large number/proportion of undescribed species, and too few genera have been recently revised or monographed to provide authoritative identifications (requiring the examination and dissection of types, e.g., Vilela and Bächli, 1990); and (3) the goal of the study is to examine overall species diversity and abundance, not identity. Species identities will eventually provide further dimensions to the project (e.g., distributions).

Basic data is presented (i.e., species abundance graphs); rarefaction analysis was not done. Rarefaction is a routine method in ecology (e.g., Gotelli and Colwell, 2001), comparing estimates of species richness based on the slopes of curves resulting from plots of numbers of taxa and samples. Rarefaction curves typically rise steeply and then asymptote, rarely leveling off (at least in insect studies). It is a comparative method. For our study, the only meaningful comparison would have been another yearlong effort using the same trapping methods, but in one or preferably several other forests. The method also works best if there is not much skew in species abundance and rarity; in our study there was profound skew. Also, rarefaction does not measure abundance and rarity, two important variables studied here.

Specimens are to be housed in the Museo Nacional de Costa Rica, San José; the Los Angeles County Natural History Museum; and the American Museum of Natural History.

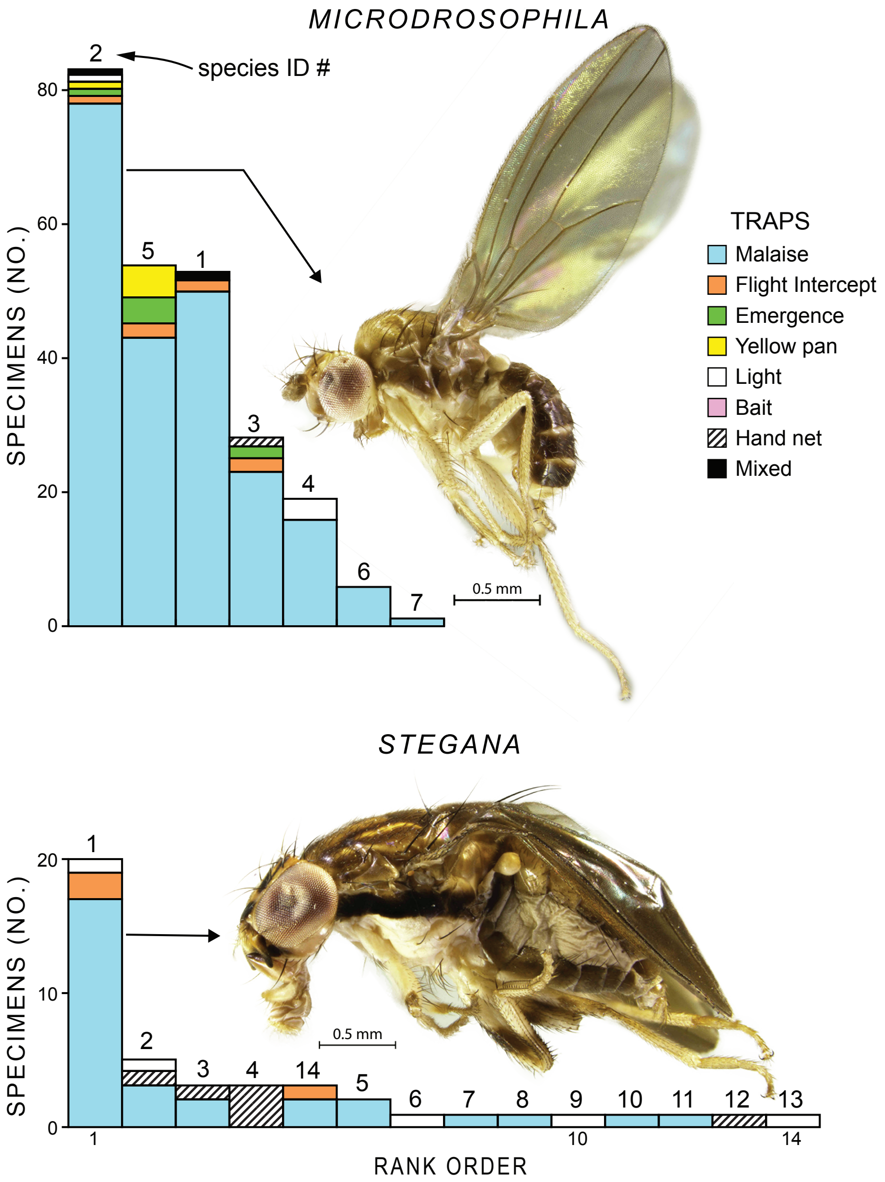

FIG. 3. Species abundances of two genera of Drosophilidae at the Zurquí study site, *Microdrosophila* (above), and *Stegana* (below), with photos of representative species. Histograms in figures 3–9 are color coded according to type of collecting method, shown in the key. Y axis shows the number of specimens; order along the X-axis is by rank in species abundance; numbers above each histogram bar are species numbers.

RESULTS

A total of 352 species in 16 described genera of Drosophilidae were found among a total of 2908 specimens (table 1). Ten species were unassignable to genus and will require further study.

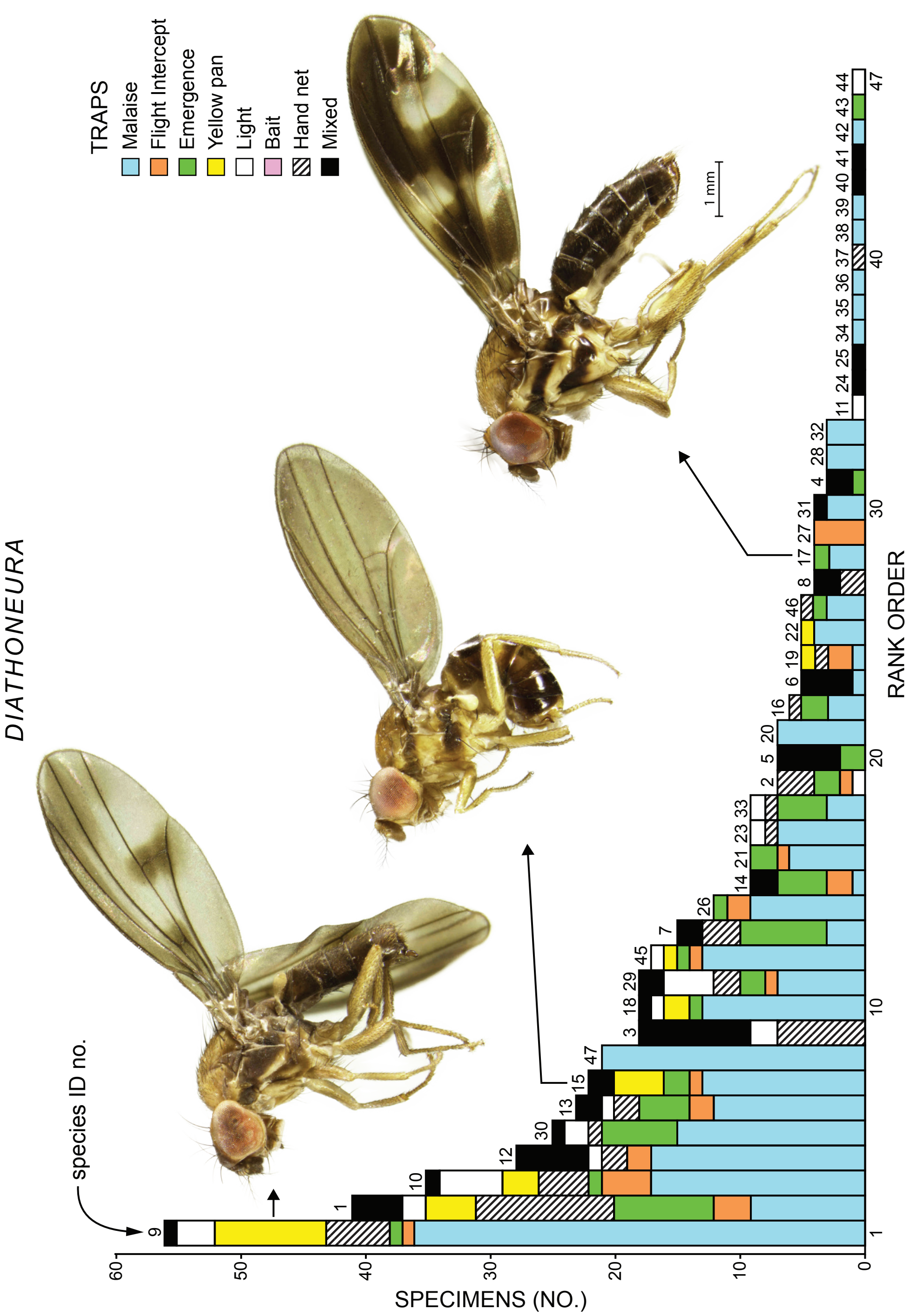

FIG. 4. Species abundances by various collecting methods of the neotropical genus *Diathoneura*.

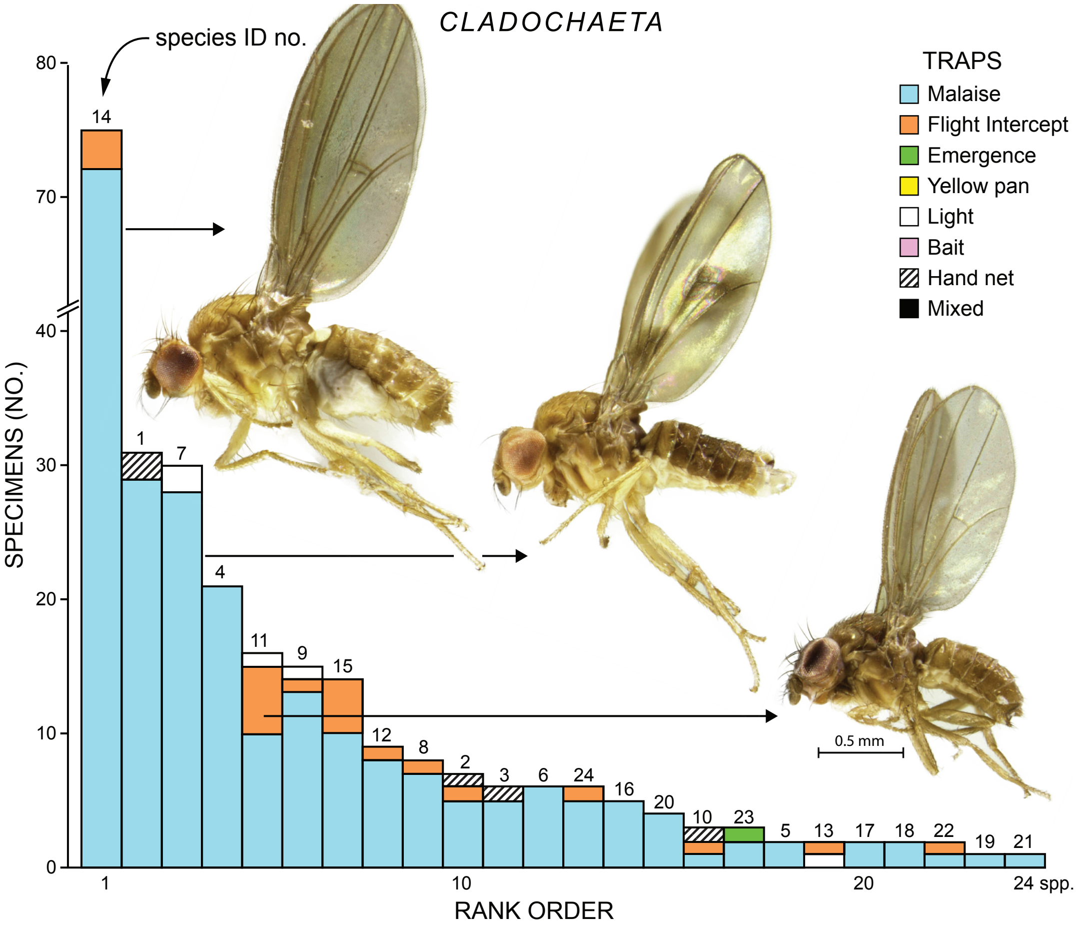

FIG. 5. Species abundances by various collecting methods of the neotropical genus *Cladochaeta*.

Missing Genera

Six genera native to the neotropics were not found, but are known to occur throughout Costa Rica: *Amiota* and *Phortica* (*Sinophthalmus*), the two genera of which are closely related (there are several subgenera of *Phortica*). There are approximately 50 Nearctic species of *Amiota* (Jones and Grimaldi, 2022); neotropical species have hardly been studied. There are only two described species of *P.* (*Sinophthalmus*) but perhaps 10 undescribed species, all from Central America. Flies in both groups are easily collected since they (especially males) are attracted to perspiration and eyes of various mammals, including humans. They prefer montane forest, where they breed in decaying trees like *Stegana* (e.g., Jones and Grimaldi, 2022). Likewise absent were *Palmomyia* Grimaldi and *Apachrochaeta* Duda, two small, closely related genera, the former of which is attracted to (and probably breeding in) palm flowers, habits of the latter genus unknown. No palms occurred at the Zurquí site. Lastly, no specimens were found in the sister genera *Pseudiastata* and *Rhinoleucophenga*, all 37 species of which are neotropical; larvae of both genera are predaceous, principally on scale insects (Ashburner, 1981); they seem to prefer open habitats like grasslands and marshes dominated by monocots (Poppe et al., 2014).

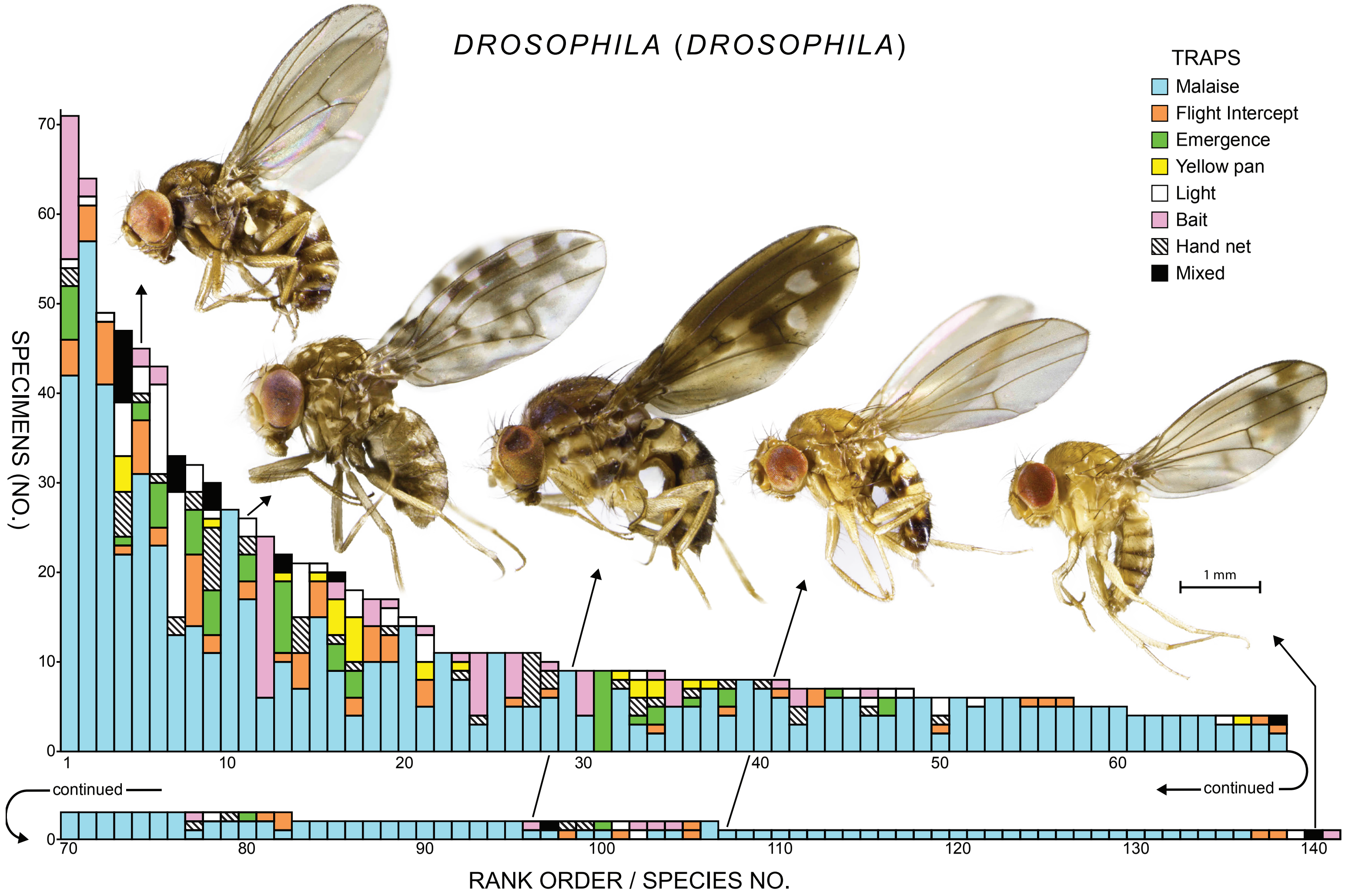

FIG. 6. Species abundances by various collecting methods of *Drosophila*, subgenus *Drosophila*. Abundances for species 1 and 2 were much higher since this graph includes only specimens that were mounted from the bait traps, not all specimens.

Groups with Low Diversity

Twelve species represented by just 40 specimens were collected from the following six genera (table 1). Two species with three specimens were captured of the sister genera *Chymomyza* + *Neotanygastrella* (there are 26 species in the New World). These can be abundant at tree wounds and damaged bark. *Leucophenga* is a global genus with several hundred species (mostly mycophagous), but only several of them occurring in the neotropics; three species represented by four specimens were collected at Zurquí. *Scaptomyza* is another global genus with hundreds of species (these are mostly leaf and stem miners); 24 specimens in four species were collected at Zurquí. Nine specimens representing three species of the flower-breeding sister genera *Laccodrosophila* + *Zapriothrica* were collected.

Groups with Significant Diversity

Microdrosophila (fig. 3A): This is a global genus of 78 described species, one in the New World. Preliminary studies by the senior author, using dissections of male genitalia of specimens throughout the Caribbean and Central America, indicates that there probably are hundreds of Neotropical *Microdrosophila* species. The genus is poorly studied because of their generally small size, external similarity, complex male genitalia, and because they have not yet been cultured in the lab. Also, they occur sporadically. We have found them in abundance by sweeping over the leaves of freshly fallen trees, although abundant specimens have also been collected elsewhere by canopy fogging. It is very likely that the larvae feed on leaves, either fresh or in incipient stages of decay, which is why they are categorized as "phytophagous" for this study.

Seven species were found in Zurquí, all of them in Malaise traps. Very modest numbers of five of the seven species were collected by other methods; 88% of the 245 specimens were collected in Malaise traps. Unlike any of the other genera/groups, the frequency distribution of *Microdrosophila* is linear—an evenly graded distribution in abundances—rather than the typical concave-curvilinear distribution where one or two species greatly predominate. The relative abundance of *Microdrosophila* in Zurquí Malaise traps is unusual; typically they are sparse in these traps in the neotropics.

Stegana (fig. 3B): This is a genus of modest sampling at Zurquí, and one that is always difficult to collect. Only 43 specimens were collected for this study; eight of the 14 species are based just on one specimen each, another four species based on three to four specimens each; *Stegana* sp. 1 predominated with 20 individuals (none of them collected by hand). Of the 272 world species of *Stegana*, only 24 are known for the neotropics. There are clearly many more neotropical species, but they will need to be systematically collected using Malaise traps (nine species were collected this way at Zurquí, five of them uniquely), hand-netting (four species collected, two uniquely), and at light traps (three species, all uniquely and with a single fly each). Canopy fogging, done at other sites, also yields substantial diversity of these flies though never in significant abundance.

Stegana are saproxylic flies, based on records of them having been reared from decaying logs/tree trunks (Jones et al., 2021; Stokland and Meyke, 2008; Teskey, 1976), and how the

TABLE 1. Summary of Drosophilidae diversity and abundance at Zurquí.

Genus (Subgenus)	No. species	No. specimens	Breeding sites
Chymomyza	1	1	tree wounds
Cladochaeta	24	271	arthropod parasites
Diathoneura	44	479	leaf litter/flowers
Dros. (*Drosophila*)	141	659	fruits, flowers, fungi, leaf litter
Dros. (*Sophophora*)	16	551	decaying fruits, leaf litter
Hirtodrosophila	12	38	fungi
Laccodrosophila	2	5	flowers
Leucophenga	3	4	fungi
Microdrosophila	7	245	wilting/decaying leaves
Mycodrosophila	1	1	fungi
Neotanygastrella	1	2	tree wounds
Paraliodrosophila	2	64	fungi
Paramycodrosophila	6	23	fungi
Scaptomyza	4	24	stem/leaf miners
Stegana	13	43	under bark
Zapriothrica	1	4	flowers
Zygothrica	58	449	fungi, flowers
Genera Indet.	10	24	?
Genus X	4	21	fungi
TOTALS	**352**	**2908**	

adults are generally collected. They are best hand-netted using a long-handled net to sweep quickly up tree trunks and under large overhanging branches, particularly dying and decaying trees. The larvae probably live subcortically on fungus. Adults are also found resting underneath leaves that overhang trails and streambeds. Their flight is quick, generally looping back to the original resting site. *Stegana* have bold body patterns (sometimes on the wings) and are easy to separate to species externally. Having more than half the total neotropical species number found at Zurquí, plus the rarity of most species, indicate that there probably are hundreds of *Stegana* species from South and Central America.

Diathoneura (fig. 4): An entirely neotropical genus of 39 described species, but clearly much larger given that 47 species were found at Zurquí alone. The senior author has always found them in abundance in neotropical forests by sweeping over leaf litter, particularly thick, wet leaf litter near the edges of streams during dry seasons. New data presented here confirms that a substantial number of species breed in leaf litter/decaying vegetation: nearly half of the species (21 of 47 species) were captured in emergence traps erected over leaf litter, branches, and even over narrow streams (*Diathoneura* larvae are certainly not aquatic, but probably feed in wet humus and leaves snagged in streams or along the edges). This abundant resource would also account for the abundance of individuals (470 specimens), although some species of *Dia-*

thoneura breed in living flowers (Pipkin et al., 1966). The genus is categorized for this study as "saprophagous" and "anthophagous."

Not surprisingly, Malaise traps captured the bulk of *Diathoneura* species (34, or 72% of the species), merely 10 species were captured *only* in Malaise traps. In fact, *Diathoneura* was collected using a greater variety of methods than were any of the other drosophilid groups in this study except the subgenus *Drosophila*: besides the 21 species (44%) from emergence traps, there were eight species (17%) in yellow pan traps, 12 species (25%) at lights, 14 species (29%) in flight-intercept traps, and at least 17 species (36%) sweep-netted (this is a minimum, many of the species obtained by "mixed methods" were also swept, among other methods). The species-abundance relationship is a rather typical one: eight species comprise more than half (57%) of all *Diathoneura* individuals; 13 species comprise a total of just 4% of all individuals.

Cladochaeta (fig. 5). This is the sister genus to *Diathoneura*, and possibly even phylogenetically within *Diathoneura*. Life histories are very different from *Diathoneura*, perhaps accounting for the differences between the two groups found here in species abundances and collection methods.

Cladochaeta is endemic to the New World, with four Nearctic species and 138 described species being Neotropical. They are difficult to study for four reasons: (1) they are minute, some are among the most minute drosophilids (one mm body length or slightly less); (2) there are few external features for sorting species; (3) species sorting/identification requires male genitalia, which are complex; and (4) these flies are never found in large series, even in Malaise traps where they are captured most, or, for those species where it is possible, by rearing. Most of the life histories are unknown, but about 10 species have been found to be larval parasites (not parasitoids) of spittlebug nymphs (Cercopidae, Aphrophoridae: Auchenorrhyncha) (Grimaldi and Nguyen, 1999; Nguyen, 2001; Pirani and Carvalho-Filho, 2019). One species in Brazil has been bred from egg sacs of a theridiid spider (Carvalho-Filho et al., 2018), which suggests that this genus could have diverse arthropod hosts.

Twenty-four species of *Cladochaeta* were found at Zurquí based on a total of 271 specimens. Interestingly, using Malaise trap samples from Zurquí from the early 1990's, provided by Paul Hanson, 11 species had previously been reported (Grimaldi and Nguyen, 1999), but this was based on far fewer Malaise samples. Thirteen additional species are now known from Zurquí, attributed to more extensive sampling in the present study.

The species-abundance distribution is a shallow, concave curve. Eleven (45%) of the species have five or fewer specimens; *Cladochaeta* species 14 comprised 27% of all specimens. Just four species (nos. 1, 7, 4, 14) totaled 157 specimens, or 57% of all individual *Cladochaeta*. All species were intercepted by Malaise traps; 10 of these were also captured using flight-intercept traps; only five specimens of four species were found by sweeping.

Grimaldi and Nguyen (1999) estimated that there may be as many as 800 species of *Cladochaeta* in the neotropics. With the sister genus *Diathoneura* (also Neotropical), this is clearly the largest lineage of Drosophilidae endemic to the New World, with probably over 1000 species combined.

Drosophila

Subgenus *Drosophila* (fig. 6): As expected, the number of *Drosophila* individuals was larger than for any other group in the study, some 659 specimens. Neotropical *Drosophila* are very diverse and very abundant at fallen fruits and flowers, on leaf litter and forest fungi. Although specimens were not databased as such, species groups represented in the samples include the *annulimana*, *bromeliae*, *calloptera*, *canalinea*, *cardini*, *castanea*, *flavopilosa*, *guarani*, *mesophragmatica*, *onycophora*, *repleta*, and *tripunctata* groups, as well as many ungrouped species.

The most abundant species were captured by diverse methods. For example, the 20 most abundant ones were captured using an average of four to five sampling methods, the next 20 an average of 2.8 sampling methods. Seventy percent of the rarest species (<4 specimens) were captured only in Malaise traps.

There were four very surprising results. First, species diversity is far greater than expected. With 141 species, *Drosophila* is 2.4× more diverse than the second-most diverse group at Zurquí, *Zygothrica* (see below). Many of the species were rare: 81 species (57%) are represented by five or fewer specimens. Possible explanations for such rare diversity are provided in the Discussion.

Second, Malaise traps captured *all but six* of the 141 species, and nearly half (68 species) were collected exclusively in Malaise traps. Conversely, bait traps captured only 25 species (17%), as well as a small proportion of the individuals. It needs to be emphasized that only a small portion of the two most abundant *D. (Drosophila)*, sp. 1 and sp. 2, were mounted, so their total abundance is undercounted here (fig. 6). This is, nonetheless, a stark example of how many *Drosophila* species are missed by using just the standard collecting method of drosophilists, bait trapping. This explains the relatively low diversity of *Drosophila* in other surveys that used bait trapping exclusively (e.g., Monteiro et al., 2016; Duarte et al., 2018).

Third, a significant number of species were captured at light traps (28, or 19%) and at emergence traps (19 species, or 13%) (nine species were captured at both). For species captured at lights there appeared to be a strong relationship between overall abundance of a species and the number at light: some of the more common species had larger proportions of individuals at light—a relationship that did not seem to pertain to the emergence-trap samples. Species in emergence traps were probably breeding in humus, on mycelia, or fallen, decaying fruits and flowers.

Fourth, there were no specimens of the invasive *Drosophila* species that are so common worldwide, despite the Zurquí site being at the very edge of disturbed land and partially surrounded by pasture. Invasives found in abundance in Costa Rican lowlands include *D.* (*Sophophora*) *melanogaster*, *simulans*, *malerkotliana*, and *suzukii*, as well as *D.* (*Drosophila*) *immigrans*, *hydei*, and *repleta.* The wet, misty, cool weather at Zurquí probably limits the altitudinal dispersal of these species.

Subgenus *Sophophora* (fig. 7): This group is taxonomically treated as a subgenus of the genus *Drosophila*, even though *Sophophora* and the nominal subgenus are not closely related based on both morphology (Throckmorton, 1962, 1975; Grimaldi, 1990a) and genetics (e.g., Finet et al., 2021). *Sophophora* is a monophyletic group, as are the two species groups in it that are endemic to the neotropics (the *willistoni* and *saltans* groups).

Species of *Sophophora* are classified into seven major species groups and several smaller ones. The center of diversity is the paleotropics, especially Southeast Asia and New Guinea, where hundreds of species in the *melanogaster* and *montium* species groups occur. Neotropical diversity is relatively modest, with three species groups (two of them endemic): *willistoni* group (neotropical only; 24 species total, 12 in Central America), *saltans* group (neotropical only, 23 species total, 10 in Central America), and the *obscura* group (a mostly Holarctic group of 45 species, five of them in Central America). All three groups have been intensively surveyed for studies in evolutionary biology (e.g., Lakovaara and Saura, 1982; Ehrman and Powell, 1982; Roman et al., 2022), so there appear to be few undescribed Neotropical species. The *willistoni* and *saltans* groups are sister groups; species in both groups breed in fallen, decaying fruits. Some species in the *willistoni* group can be very abundant at fruit falls in neotropical forests (e.g., *Drosophila nebulosa*); *saltans* group flies are rarely abundant. No *obscura* group specimens were collected at Zurquí despite the appropriate elevation; these flies prefer the higher, cooler altitudes like Zurquí. Of a total of 27 species of *Sophophora* known for Central America 16 were found at Zurquí.

Sophophora were extremely abundant at Zurquí (N = 551 specimens), but this is due to two very abundant species (spp. 1 and 2: fig. 7), which comprised 72% of all *Sophophora* specimens. Again, it needs to be emphasized that only a small portion of these two species were mounted, so the abundances of these species are underestimates. The remaining 14 species ranged from 40 individuals to one (mean of 10 specimens each). Four species are in the *saltans* group. The abundance distribution of this group, as a result, has an extremely steep drop to the base of the tail (fig. 7). A very unexpected result was how few *Sophophora* specimens were captured in fruit-baited traps (only 4% of the individuals in three species), and how many were collected in Malaise traps (60% of the individuals in all species), as well as in emergence traps (11% of individuals belonging to seven species), and by sweeping (19%, six species). The finding of seven species in emergence traps is entirely unexpected, since the widespread view is that species of *Sophophora* breed in decaying fruits, not leaf litter or humus.

Mycophagous Genera (fig. 8): The data from Zurquí have been combined in the histogram for five genera: *Hirtodrosophila* (176 world species, 28 Neotropical, 12 at Zurquí), *Mycodrosophila* (132 world, 12 Neotropical, one at Zurquí), *Paraliodrosophila* (five species, all Neotropical, two at Zurquí), *Paramycodrosophila* (16 world species, four Neotropical, six at Zurquí), and an undescribed genus, "X," which is being described in a separate paper (Grimaldi et al, in prep.). These form a well-defined monophyletic group (Grimaldi, 1990a, 1990b; Finet et al., 2021), which feed, mate, and breed in macrofungi (Grimaldi, 1987; Valer et al., 2016; Policha et al., 2019; Santa-Brígida et al., 2019). The Zurquí sample is rather modest (147 specimens, belonging to 26 species total). *Zygothrica* is also in this group of genera (Grimaldi, 1990a), but it is treated separately because it was so abundant and diverse at Zurquí.

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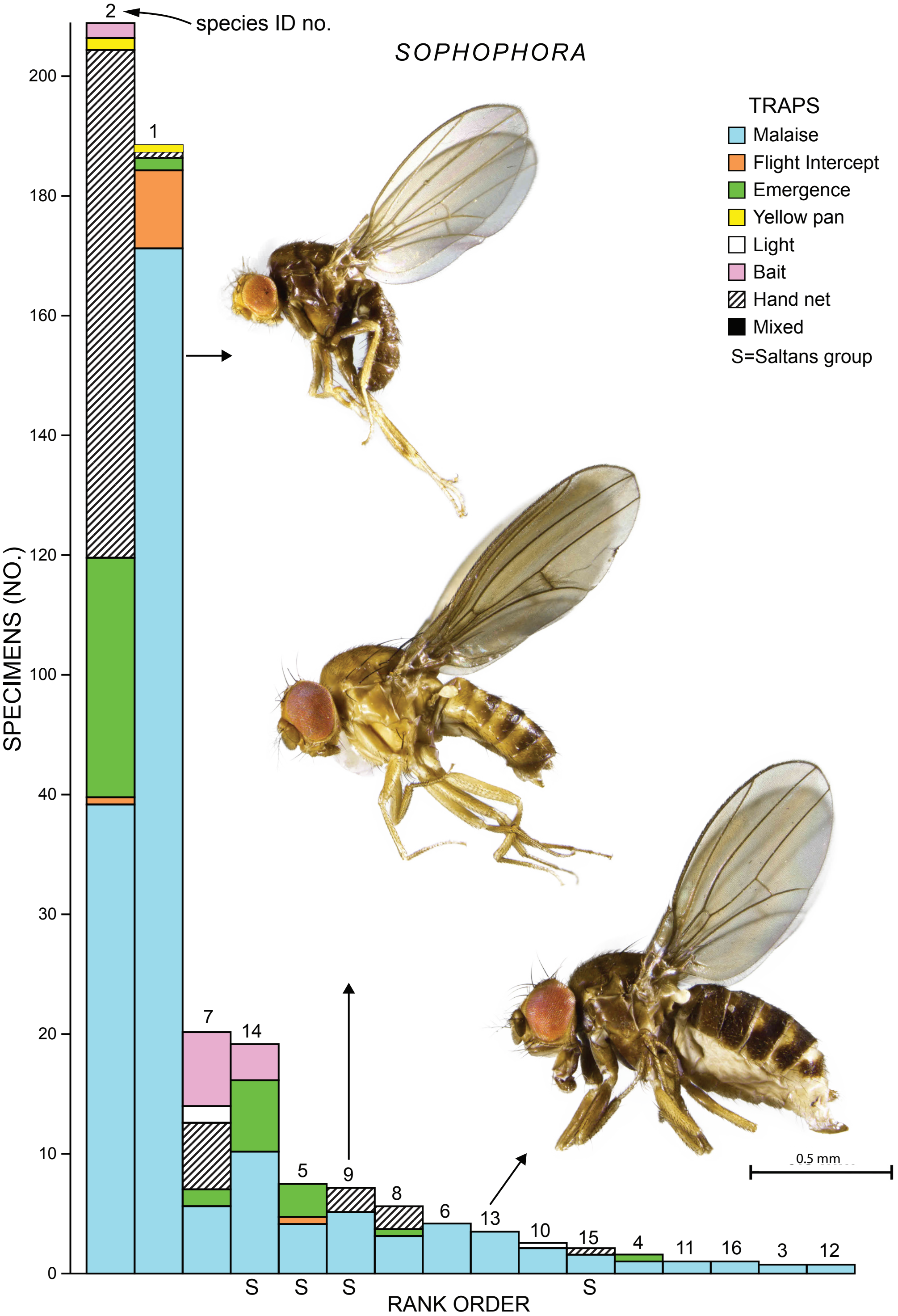
FIG. 7. Species abundances by various collecting methods of *Drosophila*, subgenus *Sophophora*. Abundances for species 1 and 2 were much higher since this graph includes only specimens that were mounted from the bait traps, not all specimens.


species ID no.
SOPHOPHORA
TRAPS
Malaise
Flight Intercept
Emergence
Yellow pan
Light
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Hand net
Mixed
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RANK ORDER
0.5 mm

Sampling these flies at Zurquí involved most of the techniques employed: Malaise traps (20 of the 26 species), hand collecting (eight species), flight-intercept traps (eight species), emergence traps (seven species), and light traps (three species). Malaise traps did not capture a substantial majority of specimens; in fact, six of the 26 species were captured only by other means. We are inclined to believe that adults of these mycophagous species do not disperse far and may settle around a site of fungus for much of their brief lives. Collecting by hand yielded most of the specimens, and these were comprised largely (43% of individuals) of *Paraliodrosophila* spp. 1 and 2. The flies collected by hand were caught on *Auricularia* (Auriculariaceae), *Ganoderma* (Polyporaceae), and an undetermined genus of polypore. The undescribed genus "X" is known to feed on, and probably breed in, particular genera of ascomycetes, based on extensive observations elsewhere. The Zurquí data reinforce our impression that thorough sampling of these genera requires collecting them on their hosts. *Hirtodrosophila* has what appears to be the largest numbers of undescribed Neotropical species in this group of genera after *Zygothrica*.

Zygothrica (fig. 9). This is a circumtropical genus currently of 125 described species, only 10 of which are Old World, all others being Neotropical. A total of 58 species were collected at Zurquí, making this the second-most diverse genus there after the subgenus *Drosophila (Drosophila)*.

Species of *Zygothrica* are morphologically and ecologically very diverse, as well as speciose. The revisions by Grimaldi (1987, 1990b) cover only part of the genus; large sections still need work. Species are small to large bodied, many with striking patterns: wing spots and other markings, notal and frontal stripes, and bands, spots or other patterns on the abdomen. There can be striking sexual dimorphism in head shape (e.g., the *dispar* group), or coloration (e.g., the *aldrichi* group).

Adult *Zygothrica* are usually found in large aggregations on forest mushrooms, where they graze, court and mate. Some species breed in the mushrooms (Grimaldi, 1987; Valer et al., 2016; Policha et al., 2019); other species (even ones congregating at fungi) breed in flowers (Grimaldi, 1987; Santa-Brígida et al., 2019; dos Santos and Vilela, 2005; Vaz et al., 2014). The attraction of *Zygothrica* to mushrooms is exploited by some pleurothalidiine orchids such as the genus *Dracula*, which lure the flies to the orchid's labellum, which looks and smells like a small mushroom (Endara et al., 2010; Policha et al., 2016, 2019).

Females of some *Zygothrica* species have impressively long, piercing oviscapts that are used for inserting eggs into flowers (fig. 9: spp. 12, 13, 23). While a long, piercing oviscapt is always associated with drosophilid species that breed in flowers (Brncic, 1983; Grimaldi 1990a), there are many anthophagous species with the typical, short oviscapt (such as the *Drosophila bromeliae* group [Grimaldi, 2016]). At Zurquí, at least 10 (17%) of the 58 species have a long, piercing oviscapt, but since some species are known only by males, and some with a typical oviscapt are probably also flower breeders, we conservatively estimate about 30% of the Zurquí *Zygothrica* are anthophagous.

The species-abundance curve for Zurquí *Zygothrica* is strongly concave, with just four of the 58 species (spp. 1, 4, 13, 19) comprising 50% of all individuals. Equally striking is the very long tail of rare species: 46 of the 58 species are known by just five or fewer specimens, suggestive of immense diversity. Indeed, there are probably hundreds of new species, especially in montane

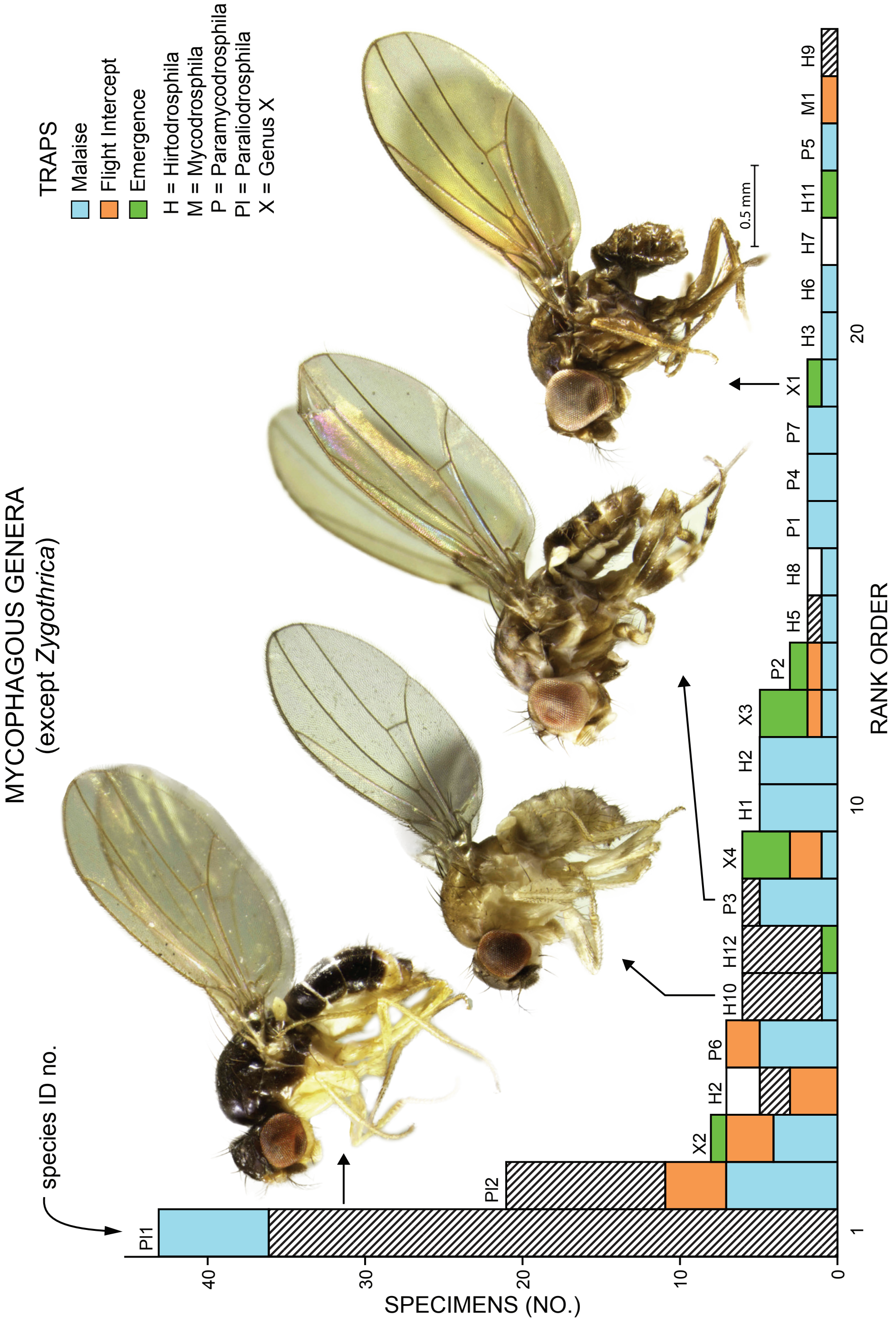

FIG. 8. Species abundances by various collecting methods of four related mycophagous genera: *Paraliodrosophila* ("Pl"), *Hirtodrosophila* ("H"), *Paramycodrosophila* ("P"), and undescribed genus ("X"). The related genus *Zygothrica* is graphed separately (Fig. 9).

neotropical forests, but it will require long-term sampling to find them. Exceptional diversity of *Zygothrica* is also suggested by a dramatic separation of species that occur in lowland rainforests and those in montane forests. At Zurquí, there were no specimens in the *Zygothrica aldrichi* and *dispar* groups, which dominate in lowlands (Grimaldi, 1987). Malaise traps were very effective at sampling *Zygothrica* at Zurquí: 84% of the individuals and all but nine of the species. Flight intercept traps sampled just 9% of the individuals and 19 of the species (five of the species uniquely: spp. 56, 59, 60, 61, 62). One specimen each in three species was obtained using emergence traps; these may have been breeding on mycelia or depleted fungal sporocarps.

GENERAL RESULTS AND DISCUSSION

Table 2 summarizes the number of species captured using the different collecting techniques. Malaise trapping (fig. 2A) was extraordinarily effective, capturing 309 (87%) of the 352 drosophilid species. Moreover, 145 (41%) of the 352 species were captured only in Malaise traps. The effectiveness and popularity of this method of trapping, especially for Diptera, has been known for decades (Brown, 2005; Borkent and Brown, 2015; Brown et al., 2018; Karlsson et al., 2020; Amorim et al., 2022). The only drawbacks with the technique are that traps should be up for at least several weeks (preferably several months) and emptied regularly, and that a great deal of bycatch is obtained.

Flight intercept trapping (FIT) (fig. 2B), not surprisingly, was the second most effective method, having captured 103 (29%) of the 352 drosophilid species, 11 species uniquely. FITs function like Malaise traps, but these capture flies that fall into the preservative trough when they hit the wall of screening, rather than species that fly upward. Genera with high numbers of species caught in Malaise traps are also captured in FITs.

Bait trapping, very surprisingly, captured just 7% of the Zurquí species (table 2), and quantifies the ineffectiveness of the most common method for general collecting of drosophilids. It draws an abundance of certain *Drosophila*, but relatively few species. Bait trapping is probably why several studies on drosophilids in Brazil reported many fewer species (e.g., 55 species: Monteiro et al., 2016; 37 species: Duarte et al., 2018), although the study by Medeiros and Klaczko (2004) used this technique and found 125 species among nearly 30,000 specimens, sampled from three isolated areas of Atlantic forest in the state of São Paulo.

Emergence traps (figs. 2C, D) have particular significance because they document some of the species that are breeding residents of the Zurquí study site, in this case 65 (18%) of the 352 species. Adding to this a further seven mycophagous species, which were captured by hand on fungi at Zurquí, then at least 72, or 20%, of the Zurquí species are resident. It is very plausible, even likely, that 50% or more of the species (~175 species) captured at this site are breeding residents or part of a biological community of species that interact directly or indirectly with each other. The number and species composition of residents of a biological community fluctuate around a coarse equilibrium, with some local, ephemeral extinction and colonization steadily taking place (Jacquemyn et al., 2001; Ricklefs and Schluter, 1983). While the presence of *Diathoneura* in emergence traps was not surprising, their abundance and diversity was:

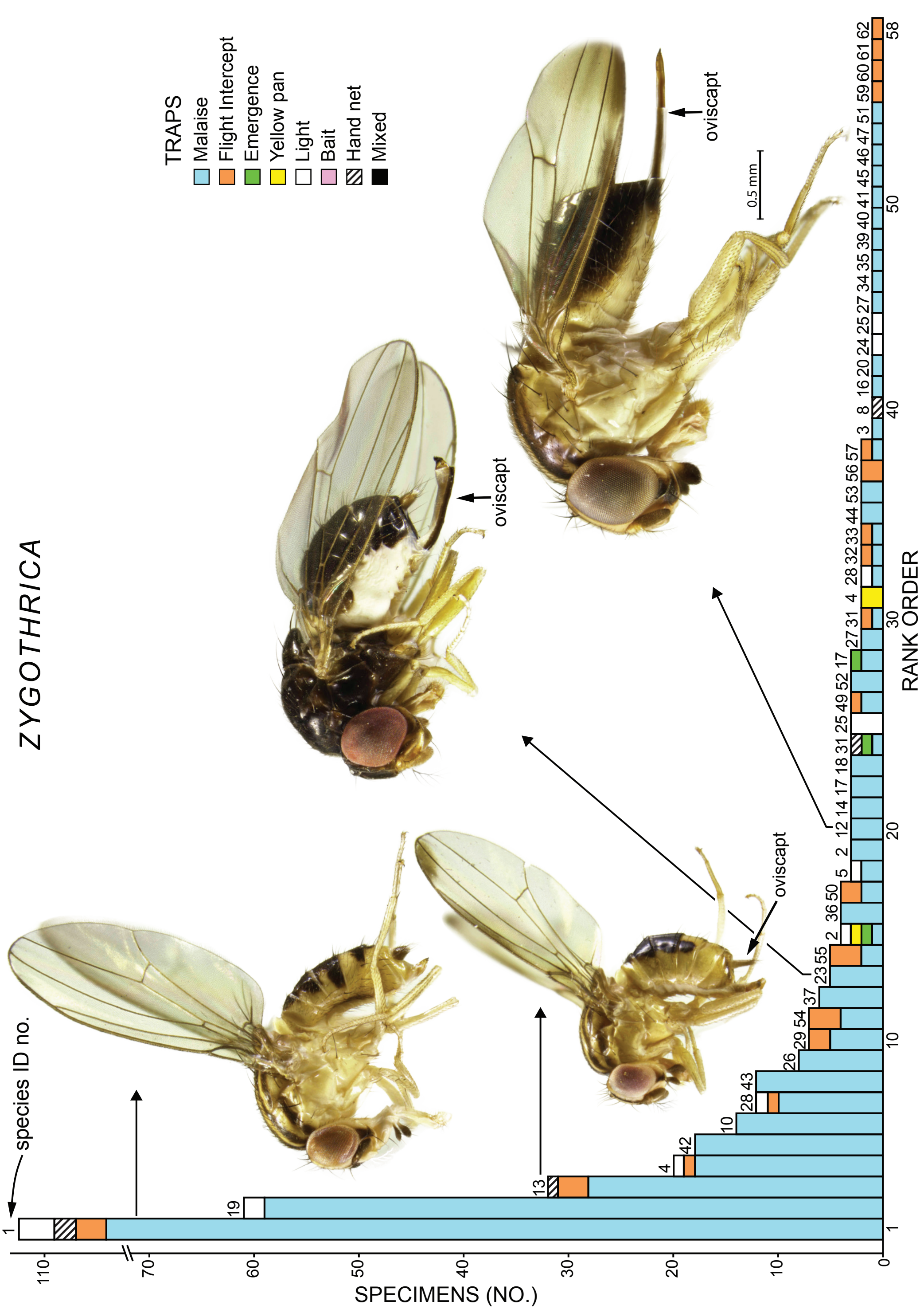

FIG. 9. Species abundances by various collecting methods of the genus *Zygothrica*. The long oviscapts for species 12, 13, and 23 indicate they are flower-breeding species.

nearly half of the *Diathoneura* species (21 of 47 species) were captured in emergence traps, establishing that this genus is significantly saprophagous, the larvae probably grazing on humus, mycelia, and microflora in the soil and leaf litter. The genus is commonly assumed to be largely anthophagous (e.g., Pipkin et al., 1966). Even more surprising was the finding that 26 species of *Drosophila* (in both major subgenera) were breeding in leaf litter.

ABUNDANCE AND RARITY: Abundant species were arbitrarily defined for this study as ones with 50 or more specimens. There were only 11 abundant species, comprising 1030 or 35% of all specimens in the study (an underestimate of true abundance for five of these species). The abundant species were not all from species-rich genera. For example, there were three abundant species of *Microdrosphila* (for which only seven species were collected), and two abundant species each for *Drosophila* (*Drosophila*) and *Zygothrica* (with 141 and 58 species, respectively). *Diathoneura* and *Cladochaeta* each had one abundant species; *D. (Sophophora)* had two.

Fully two-thirds (66%, or 234) of the species found in this study were rare, represented by five or fewer specimens. There are high proportions of rare insect species in diverse biological communities. Morse et al. (1988), for example, collected 859 beetle species in a lowland forest in Borneo using insecticidal fogging, 496 (57%) of which had one specimen each and only 10 species had more than 100 individuals.

Rarity, actual or apparent, can be due to various factors, either singly or in various combinations; some of these correspond to the categories in the classification by Rabinowitz (1981):

1. Habits of a species may not readily allow interception at or attraction to traps. This seems to be the case, for example, with some *Stegana*, which are best surveyed by sweeping a net along tree trunks and overhanging vegetation. Species in the canopy or higher in the forest would probably be less likely to be trapped near the ground. Vertical stratification is well known in drosophilids and various flies (e.g., van Klinken and Walter, 2001b; Roque et al., 2013; Amorim et al., 2022). In the study by Amorim et al. (2022), 60% of the species did not occur at ground level, making vertical stratification potentially a major source of undersampling.
2. A species may have a brief, seasonal flight period, which would lower its probability of capture.
3. Probably all species of plants and animals experience multiyear population fluctuations, which can be dramatic in some years. The capture of at least some rare drosophilid species here may have coincided with low periods in their population cycles.
4. Some of the rare species could be at the edges of their geographical distribution at Zurquí or in marginal habitat. Species would need to be identified and their distributions plotted to assess for distributional edge effects.
5. In converse to 4 above, a rare species could indeed be very rare, with a very small, highly endemic distribution. Or, rare species may have very specific niches that are sparse or nonexistent at the Zurquí study site. This is probably the case with many of the flower-breeding species of *Drosophila*, *Zygothrica*, and small

TABLE 2. Number of species captured by sampling method (taxa in alphabetical order). Methods: **MT**, Malaise trap; **FIT**, flight intercept trap; **EM**, emergence trap; **YP**, yellow pan trap; **LT**, light trap; **BT**, bait trap; **HD**, hand netted; **MX**, mixed methods.

Genus	**MT**	**FIT**	**EM**	**YP**	**LT**	**BT**	**HD**	**MX**
Chymomyza	2	0	0	0	0	0	0	0
Cladochaeta	23	11	1	0	4	0	4	0
Diathoneura	33	14	21	8	15	0	17	21
Dros (Drosophila)	131	35	19	13	27	25	29	8
Dros (Sophophora)	16	3	7	2	1	3	5	0
Laccodrosophila	2	0	0	0	0	0	0	0
Leucophenga	1	1	1	0	1	0	0	0
Microdrosophila	7	4	3	2	2	0	1	2
Mycoph. Genera	20	8	7	0	3	0	8	0
Neotanygastrella	1	0	0	0	0	0	0	0
Scaptomyza	4	1	1	0	0	0	0	0
Stegana	9	2	0	0	5	0	4	0
Zapriothrica	1	1	0	0	0	0	0	0
Zygothrica	54	18	3	2	10	0	4	0
Genera indet.	5	5	2	0	0	0	0	0
TOTALS	309	103	65	27	68	28	72	31
Proportion of 352 spp.	.87	.29	.18	.07	.19	.07	.20	.08
Unique spp. (no.)	145	11	3	1	10	1	5	5

genera like *Laccodrosophila* and *Zapriothrica*, especially since some anthophagous drosophilid species can be very host specific (Brncic, 1983).

6. Random dispersal probably accounts for some rarity in any area, with individuals that are itinerants passing through but are not breeding residents. Drosophilids disperse surprisingly well. Mark-recapture studies have determined daily movement of 100–300 m per day for several species in the *Drosophila* (*S.*) *obscura* group (Taylor et al., 1984), and at least a kilometer per day for a desert species in the *repleta* group (Johnston and Heed, 1976). With such dispersal ability itinerant drosophilids can pass through the study site in less than a day, occasionally being intercepted. Likewise, strong winds can waft flies into an area.

Global Comparisons and Tropical Diversity

Table 3 summarizes global comparisons with some countries/areas that have been well explored. Not included were some areas like the British Isles or Japan where drosophilid faunas are well known, but which are insular and have fewer species (even for continental islands). Hawaii is unique: as of 2022 it is known to have 564 endemic species of drosophilids (33 introduced; Bächli, 2022) in just 16,636 km^2 for the entire archipelago, an area smaller than Belize. Hawaii was also not included in this comparison because as a remote, tropical archipelago with dramatic altitudinal variation, lacking many of the predators and competitors found in continental biotas, there has been extraordinary radiation of some groups. No one forested area in Hawaii, however, has 350 drosophilid species, as occur in the four hectares at Zurquí. Many species of Hawaiian drosophilids are endemic to one of the main islands (Hawai'i, Maui, Oahu, Kauai, Molokai, Lanai), and even to areas within islands.

Despite the crude comparisons in table 3 in size of areas and no doubt in collection methods, a dramatic latitudinal species gradient (LSG) is readily apparent for Drosophilidae. Countries in Europe and states in the U.S. have relatively modest numbers of species (55–72), despite their large areas, in comparison to low latitudes, consistent with the pervasive global pattern of increasing species richness toward the equator, which is not universal for all taxa (Hillebrand, 2004).

As has been known since at least von Humboldt's time, the climatic differentials between lowland and montane habitats (in temperature, moisture, etc.) is greater in tropical than temperate regions, which imposes more of a barrier to dispersal for tropical species (Janzen, 1967). This explains why tropical species in general tend to have smaller distributions, called Rapoport's rule (Stevens, 1989), and specifically why tropical drosophilids have dramatic altitudinal stratification (e.g., Grimaldi et al., 2000), including the complete lack at Zurquí of widespread invasive drosophilid species (see above, *Drosophila*). It is well established experimentally that tropical *Drosophila* have a narrower thermal tolerance than temperate ones (e.g., Kellerman et al., 2012). There is, for example, complete altitudinal separation of species in at least some Drosophilidae genera between Zurquí and the lowland rain forest at La Selva Biological Station (which are only 48 km apart): no species of *Cladochaeta* or *Zygothrica* are in common (Grimaldi, 1987; Grimaldi and Nguyen, 1999; Grimaldi, unpubl.).

Our study not only confirms that the number of described species of Drosophilidae for Costa Rica (305) is well below the actual number, but given the diversity of biomes and altitudes in Costa Rica (Janzen, 1983), a prior estimate of 500 drosophilid species for Central America (Grimaldi, 2010) is clearly too low. A revised estimate would be approximately 800 species or more in Costa Rica alone.

Huang et al. (2022) recently reported a faunal diversity of Diptera even larger than at Zurquí, from Tianmu Mountain (119°24′–27'E, 30°19′–21′N) in the eastern province of Zhejiang, China west of Shanghai. They reported 5092 species ("OTUs" [operational taxonomic units]) in 72 families, versus 4332 species in 73 families from Zurquí. That study used only Malaise traps. A difference of 760 more species at Tianmu is attributable to several differences in methods between the two studies, one of which is a sampling area for the mountain that is several orders of magnitude larger as well as being altitudinally diverse (320–1150 m). Second, the Tianmu study used metabarcoding (sequencing of Malaise trap residues), which no doubt detected morphologically cryptic diversity, but narrower species concepts may also have been applied to genetically define "OTUs." Third, most of the differences were in Cecidomyiidae and Sciaridae (1529 vs. 800 species, and 698 vs. 204 species, respectively). These two families alone account for a difference of 1223 species between Tianmu and Zurquí. At Zurquí, sorting ended

TABLE 3. Comparison of some continental drosophilid faunas (from highest to lowest). References: **1.** Bächli, 2022. **2.** Huang et al., 2022. **3.** Khali et al., 2022. **4.** Tsacas et al., 1981. **5, 6.** Patterson, 1943; Grimaldi, unpubl. **7.** Bächli et al., 2004. **8, 9.** Miller et al., 2017; Jones and Grimaldi, 2022.

Country-Region	**Area, km^2**	**Latitudinal range**	**No. species**	**Ref.**
Zurquí, Costa Rica	0.04	10.47 N	352	herein
Costa Rica (entire)	51,060	8.05–11.16 N	305*	1
China: Tianmu Mtn.	?	30°19′–21′ N	209	2
Indian Himalaya states	335,138	22.05–35.51 N	172*	3
Ivory Coast (entire)	322,463	4.42–10.67 N	137	4
Texas (entire)	695,621	25.95–36.45 N	72*	5, 6
Finland (entire)	338,455	59.83–70.04 N	61*	7
Switzerland (entire)	41,285	45.83–47.79 N	59*	1
New York State	141,297	40.50–44.98 N	55*	8, 9

* Based largely/entirely on the number of described species.

when 800 morphospecies of Cecidomyiidae were reached. Otherwise, most other families at Zurquí had more species (e.g., 286 Tachinidae vs. 116 at Tianmu; 225 Tipulidae vs. 114 at Tianmu), including Drosophilidae. Rarefaction estimated 8000 Diptera species at Zurquí, but that was based on incomplete sampling for cedidomyiids and drosophilids.

For Drosophilidae, Huang et al. (2022) reported 209 species at Tianmu Mountain (vs. 352 at Zurquí), which seems appropriate given that the drosophilid fauna of China has over 700 described species (in 9,596,000 km^2) (Bächli, 2022) (vs. 305 described species in Costa Rica [51,060 km^2]). Tianmu is humid, subtropical evergreen broad-leaved forest, with more than one meter of annual precipitation (this explains why there are nearly three times more drosophilid species at Tianmu than in Texas, even though they are about at the same latitude [table 3]). Lastly, while metabarcoding may greatly expedite insect faunal surveys, the results need to be compared to the actual specimens to assess accuracy of the method.

We compared Zurquí diversity to another highly diverse, neotropical area that has been reasonablly well sampled for drosophilids, Reserva Etnica Waorani, Orellana, eastern Ecuador (table 4). Waorani Reserve is a very large area of approximately 10,000 km^2 of lowland forest. The late Terry L. Erwin (NMNH) collected throughout the reserve in the 1990's in various habitats and microhabitats using insecticidal fogging, sprayed into forest canopies, under overhanging branches, under decaying logs, in tree holes, on understory vegetation, etc.; drosophilid specimens were extracted from the insect residues. We selected the following genera for comparison: *Cladochaeta*, *Stegana*, and six genera in the mycophagous group of genera (*Hirtodrosophila*, *Mycodrosophila*, *Paraliodrosophila*, *Paramycodrosophila*, *Zygothrica*, and undescribed genus "X"). Comparison between the two sites is very uneven because of disparate collecting methods, but still informative. Waorani Reserve had 150 species in these seven genera, versus 120 at Zurquí, the main contributors being *Cladochaeta*, *Stegana*, and *Hirtodrosophila*. There was less than half the number of *Zygothrica* species at Waorani as at Zurquí, which must reflect the mid-montane preferences of most species in this genus.

Waorani species diversity is probably a significant underestimate, since only one sampling method was used, albeit one that allowed roaming the entire sampling area. Sampling was also not continuous like at Zurquí, done neither throughout the year nor at night when some species fly. Lastly, the total number of specimens collected for these genera at Waorani was less than half that at Zurquí.

Although Costa Rica and Central America in general are very rich, we estimate that the highest species diversity of Drosophilidae in the world is in the lowland and mid-montane forests of the eastern Andes from Colombia to northern Bolivia. This reflects bird diversity: 508 nonvagrant species in Manu NP, Peru (largely around the lowland Cocha Cashu area), versus 363 and 383 at La Selva Biological Station in Costa Rica and Barro Colorado Island, Panama, respectively (Karr et al., 1990). Plant species diversity (which is more intimately related ecologically to drosophilids) shows a different pattern: 1370 species at Manu, versus 1668 and 1320 species at La Selva and Barro Colorado, respectively (Gentry, 1990). Further fieldwork is needed to assess our prediction.

Several factors theoretically contribute to great species richness in the tropics besides the accentuated climatic effects due to altitude: geological age of tropical forests, climatic stability (e.g., lower extinction), greater productivity, and higher speciation (Mittelbach et al., 2007). With regard to productivity and speciation, the emergence of distinctly tropical niches probably accounts for some higher species richness in tropical drosophilids, but hardly all. Obligate breeding in various fruits and flowers, for example, are niches essentially absent in north temperate drosophilids, but at Zurquí there were in these niches an estimated 25% and 15% of species, respectively. This does not account for approximately an order of magnitude more species at Zurquí than any one temperate site. Moreover, temperate forests have very abundant leaf litter and macrofungi and still are relatively depauperate in species of the mycophagous genera, in *Microdrosophila*, and they have no *Diathoneura*. Perhaps Rapoport's Rule explains the great diversity of tropical Drosophilidae, tests for which would require an analysis of species ranges. Drosophilidae provide multifaceted opportunities for addressing classic problems in ecology and biodiversity, as well as raising new questions.

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TABLE 4. Comparison of species numbers of select drosophilid genera at Zurquí, Costa Rica, and Waorani Reserve, Orellana, Ecuador.*

Genus	**Number of Species**	
	Zurquí	**Orellana***
Cladochaeta	24	43
Stegana	14	23
	Mycophagous Genera	
Hirtodrosophila	12	31
Mycodrosophila	1	8
Paramycodrosophila	7	9
Genus X	4	12
Zygothrica	58	24
TOTAL (spp.)	**120**	**150**

* Collections made using insecticidal fogging.

Waorani fogging samples and borrow drosophilids; Michael Turrelli, who provided stimulating conversation while working at Zurquí; Steve Thurston (AMNH), for providing the excellent graphics; and, lastly, our gratitude to Brian Brown and Art Borkent, who conceived of ZADBI, organized it, and obtained funding through NSF grant DEB 1145890 that paid for all aspects of this project, including a trip by the senior author to work in Costa Rica. We are grateful to Dalton Amorim and Art Borkent for their detailed, thoughtful, and very helpful commentary on the manuscript.

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