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Remarkable fly (Diptera) diversity in a patch of Costa Rican cloud forest: Why inventory is a vital science

ART BORKENT¹, BRIAN V. BROWN², PETER H. ADLER³, DALTON DE SOUZA AMORIM⁴, KEVIN BARBER⁵, DANIEL BICKEL⁶, STEPHANIE BOUCHER⁷, SCOTT E. BROOKS⁸, JOHN BURGER⁹, Z.L. BURINGTON¹⁰, RENATO S. CAPELLARI¹¹, DANIEL N.R. COSTA¹², JEFFREY M. CUMMING⁸, GREG CURLER¹³, CARL W. DICK¹⁴, J.H. EPLER¹⁵, ERIC FISHER¹⁶, STEPHEN D. GAIMARI¹⁷, JON GELHAUS¹⁸, DAVID A. GRIMALDI¹⁹, JOHN HASH²⁰, MARTIN HAUSER¹⁷, HEIKKI HIPPA²¹, SERGIO IBÁÑEZ-BERNAL²², MATHIAS JASCHHOF²³, ELENA P. KAMENEVA²⁴, PETER H. KERR¹⁷, VALERY KORNEYEV²⁴, CHESLAVO A. KORYTKOWSKI[†], GIAR-ANN KUNG², GUNNAR MIKALSEN KVIFTE²⁵, OWEN LONSDALE²⁶, STEPHEN A. MARSHALL²⁷, WAYNE N. MATHIS²⁸, VERNER MICHELSEN²⁹, STEFAN NAGLIS³⁰, ALLEN L. NORRBOM³¹, STEVEN PAIERO²⁷, THOMAS PAPE³², ALESSANDRE PEREIRA-COLAVITE³³, MARC POLLET³⁴, SABRINA ROCHEFORT⁷, ALESSANDRA RUNG¹⁷, JUSTIN B. RUNYON³⁵, JADE SAVAGE³⁶, VERA C. SILVA³⁷, BRADLEY J. SINCLAIR³⁸, JEFFREY H. SKEVINGTON^{†8}, JOHN O. STIREMAN III¹⁰, JOHN SWANN³⁹, PEKKA VILKAMAA⁴⁰, TERRY WHEELER^{††}, TERRY WHITWORTH⁴¹, MARIA WONG², D. MONTY WOOD⁸, NORMAN WOODLEY⁴², TIFFANY YAU²⁷, THOMAS J. ZAVORTINK⁴³ & MANUEL A. ZUMBADO⁴⁴

[†]—deceased. Formerly with the Universidad de Panama

^{††}—deceased. Formerly at McGill University, Canada

¹ Research Associate, Royal British Columbia Museum and the American Museum of Natural History, 691-8th Ave. SE, Salmon Arm, BC, V1E 2C2, Canada. Email: artborkent@telus.net

² Entomology Section, Natural History Museum of Los Angeles County, 900 Exposition Boulevard, Los Angeles, CA, 90007, USA. Emails: bbrown@nhm.org; gkung@nhm.org; mwong@nhm.org

³ Department of Plant and Environmental Sciences, 130 McGinty Court, E-143 Poole Agricultural Center, Clemson University, Clemson, SC, 29634-0310 USA. Email: padler@CLEMSON.EDU

⁴ Depto. de Biologia, FFCLRP, Universidade de São Paulo, Av. Bandeirantes 3900, 14.040-901 Ribeirão Preto, São Paulo, Brazil. Email: dsamorim@usp.br

⁵ Great Lakes Forestry Centre, Canadian Forest Service, Natural Resources Canada, 1219 Queen St. E., Sault Ste. Marie, Ontario, P6A 2E5, Canada. Email: kbarber@NRCan.gc.ca

⁶ Australian Museum, 1 William Street, Sydney, NSW, 2010, Australia. Email: Dan.Bickel@austmus.gov.au

⁷ Department of Natural Resource Sciences, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada. Email: stephanie.boucher@mcgill.ca; sabrina.rochefort@mail.mcgill.ca

⁸ Canadian National Collection of Insects, Arachnids and Nematodes, Invertebrate Biodiversity, Agriculture and Agri-Food Canada, K.W. Neatby Building, 960 Carling Avenue, Ottawa, Ontario, K1A 0C6, Canada. Emails: Scott.Brooks@agr.gc.ca; Jeff.Cumming@agr.gc.ca; jhskevington@gmail.com; mgwood@mac.com

⁹ Dept. of Biological Sciences, Spaulding Hall, University of New Hampshire, Durham, NH, 03824, USA. Email: John.Burger@unh.edu

¹⁰ Department of Biological Sciences, Wright State University, 3640 Colonel Glenn Hwy, Dayton, OH, 45431, USA. Emails: burlington.2@wright.edu; john.stireman@wright.edu

¹¹ Instituto Federal do Triângulo Mineiro – Campus Uberaba. Rua João Batista Ribeiro 4000, Distrito Industrial II, 38064-790, Uberaba, Minas Gerais, Brazil. Email: rscaPELLARI@gmail.com

¹² Departamento de Zoologia, Universidade Federal do Paraná, Jardim das Américas, 81531-980 – Curitiba, Paraná, Brazil. Email: negosekidan@gmail.com

¹³ Mississippi Entomological Museum, Mississippi State University, 100 Old Highway 12, P.O. Drawer 9775, Mississippi State, MS, 39762-9775, USA. Email: gcurler@gmail.com

¹⁴ Department of Biology, Western Kentucky University, Bowling Green, KY, 42101, USA and Integrative Research Center, Field Museum of Natural History, Chicago, IL, 60605, USA. Email: carl.dick@wku.edu

¹⁵ 461 Tiger Hammock Road, Crawfordville, FL, 32327, USA. Email: johnep3@comcast.net

¹⁶ Research Associate, California State Collection of Arthropods, 2683 Tam O' Shanter Dr., El Dorado Hills, CA, 95762, USA. Email: dasyllis@gmail.com

- ¹⁷ California State Collection of Arthropods, California Department of Food and Agriculture, 3294 Meadowview Rd., Sacramento, CA, 95832–1448, USA. Emails: stephen.gaimari@cdfa.ca.gov; mhauser@cdfa.ca.gov; arung@cdfa.ca.gov; peter.kerr@cdfa.ca.gov
- ¹⁸ The Academy of Natural Sciences of Drexel University, 1900 Ben Franklin Parkway, Philadelphia, PA, 19103-1195, USA. Email: gelhaus@acnatsci.org
- ¹⁹ American Museum of Natural History, Central Park West at 79th St., New York, NY, V
- ²⁰ Department of Entomology, University of California, Riverside, 900 University Ave., Riverside, CA, 92521, USA. Email: jhash001@ucr.edu
- ²¹ Zoological Museum, Biodiversity Unit, FI-20014, University of Turku, Finland. Email: heikki.hippa@gmail.com
- ²² Instituto de Ecología, A.C. (INECOL), Red Ambiente y Sustentabilidad. Carretera Antigua a Coatepec 351, Col El Haya, Xalapa, CP 91070, Veracruz, México. Email: sibanber@gmail.com
- ²³ Station Linné, Ölands Skogsby 161, SE-38693 Färjestaden, Sweden. Email: mjaschhof@yahoo.de
- ²⁴ I. I. Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine, Bogdan Chmielnicki St. 15, 01030 Kyiv, Ukraine. Email: kameneva.elena@gmail.com
- ²⁵ Department of Natural History, University Museum of Bergen, University of Bergen, P.O. Box 7800, 5020 Bergen, Norway. Email: gunnar.kvifte@uib.no
- ²⁶ Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON, K1A 0C6, Canada. Email: neoxabea@hotmail.com
- ²⁷ School of Environmental Sciences, University of Guelph, Guelph, ON, N1G 2W1, Canada. Emails: samarsha@uoguelph.ca; steve.paiero@gmail.com; tyau@alumni.uoguelph.ca
- ²⁸ Department of Entomology, PO Box 37012, MRC 169; Smithsonian Institution, Washington D.C., 20013-7012, USA. Email: mathisw@si.edu
- ²⁹ Research Associate, Natural History Museum of Denmark, Universitetsparken 15, DK-2100 Copenhagen, Denmark. Email: vmichelsen@snm.ku.dk
- ³⁰ Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. Email: s.naglis@bluewin.ch
- ³¹ Systematic Entomology Laboratory, USDA, ARS, c/o National Museum of Natural History, MRC-168, P.O. Box 37012, Washington DC, 20013-7012, USA. Email: allen.norrbom@ars.usda.gov
- ³² Natural History Museum of Denmark, Universitetsparken 15, DK-2100 Copenhagen, Denmark. Email: TPape@snm.ku.dk
- ³³ Departamento de Sistemática e Ecologia, CCEN, Universidade Federal da Paraíba, Castelo Branco, s/n, CEP 58.051-900, João Pessoa, Paraíba, Brazil. Email: alepercol@gmail.com
- ³⁴ Research Institute for Nature and Forest (INBO), Kliniekstraat 25, B-1070 Brussels, Belgium; Research Group Terrestrial Ecology (TEREC), Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium; and Entomology Unit, Royal Belgian Institute for Natural Sciences (RBINS), Vautierstraat 29, B-1000 Brussels, Belgium. Email: mpollet.doli@gmail.com
- ³⁵ USDA Forest Service, Rocky Mountain Research Station, Forestry Sciences Laboratory, 1648 S. 7th Avenue, Bozeman, MT, 59717, USA. Email: jbr160@gmail.com
- ³⁶ Department of Biological Sciences, Bishop's University, 2600 College Street, Sherbrooke, QC, J1M 1Z7, Canada. Email: jsavage@ubishops.ca
- ³⁷ Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Departamento de Morfologia e Fisiologia Animal; Via de Acesso Prof. Paulo Donato Castellane, s/n; 14884-900, Jaboticabal, São Paulo, Brazi. Email: vcsilva@fcav.unesp.br
- ³⁸ Canadian National Collection of Insects & Canadian Food Inspection Agency, OPL-Entomology, K.W. Neatby Bldg., C.E.F., 960 Carling Ave., Ottawa, ON, K1A 0C6, Canada. Email: Bradley.Sinclair@inspection.gc.ca
- ³⁹ Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, AB, T2N 1N4, Canada. Email: jeswann@ucalgary.ca
- ⁴⁰ Finnish Museum of Natural History, Zoology Unit, FI-00014 University of Helsinki, Finland. Email: pekka.vilkamaa@helsinki.fi
- ⁴¹ Washington State University, 2533 Inter Avenue, Puyallup, WA, 98372, USA. Email: blowfly@twhitworth.com
- ⁴² Research Associate, National Museum of Natural History, Smithsonian Institution; 8920 S Bryerly Ct., Hereford, AZ, 85615, USA. Email: normwoodley@gmail.com
- ⁴³ Research Associate, Bohart Museum of Entomology, University of California, One Shields Avenue, Davis, CA, 95616, USA. Email: tjzavortink@ucdavis.edu
- ⁴⁴ Research Associate, Instituto Nacional de Biodiversidad (INBio), 22-3100 Santo Domingo, Heredia, Costa Rica. Email: zzuman@gmail.com

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Abstract

Study of all flies (Diptera) collected for one year from a four-hectare (150 x 266 meter) patch of cloud forest at 1,600 meters above sea level at Zurquí de Moravia, San José Province, Costa Rica (hereafter referred to as Zurquí), revealed an astounding 4,332 species. This amounts to more than half the number of named species of flies for all of Central America. Specimens were collected with two Malaise traps running continuously and with a wide array of supplementary collecting methods for three days of each month. All morphospecies from all 73 families recorded were fully curated by technicians before submission to an international team of 59 taxonomic experts for identification.

Overall, a Malaise trap on the forest edge captured 1,988 species or 51% of all collected dipteran taxa (other than of Phoridae, subsampled only from this and one other Malaise trap). A Malaise trap in the forest sampled 906 species. Of other sampling methods, the combination of four other Malaise traps and an intercept trap, aerial/hand collecting, 10 emergence traps, and four CDC light traps added the greatest number of species to our inventory. This complement of sampling methods was an effective combination for retrieving substantial numbers of species of Diptera. Comparison of select sampling methods (considering 3,487 species of non-phorid Diptera) provided further details regarding how many species were sampled by various methods.

Comparison of species numbers from each of two permanent Malaise traps from Zurquí with those of single Malaise traps at each of Tapantí and Las Alturas, 40 and 180 km distant from Zurquí respectively, suggested significant species turnover. Comparison of the greater number of species collected in all traps from Zurquí did not markedly change the degree of similarity between the three sites, although the actual number of species shared did increase.

Comparisons of the total number of named and unnamed species of Diptera from four hectares at Zurquí is equivalent to 51% of all flies named from Central America, greater than all the named fly fauna of Colombia, equivalent to 14% of named Neotropical species and equal to about 2.7% of all named Diptera worldwide. Clearly the number of species of Diptera in tropical regions has been severely underestimated and the actual number may surpass the number of species of Coleoptera.

Various published extrapolations from limited data to estimate total numbers of species of larger taxonomic categories (e.g., Hexapoda, Arthropoda, Eukaryota, etc.) are highly questionable, and certainly will remain uncertain until we have more exhaustive surveys of all and diverse taxa (like Diptera) from multiple tropical sites.

Morphological characterization of species in inventories provides identifications placed in the context of taxonomy, phylogeny, form, and ecology. DNA barcoding species is a valuable tool to estimate species numbers but used alone fails to provide a broader context for the species identified.

Key words: biodiversity, tropical, inventory, Central America, Neotropical Region, barcoding, species richness, Cecidomyiidae, Phoridae, Tachinidae, Mycetophilidae, Drosophilidae, Sciaridae, Ceratopogonidae, Tipulidae, Dolichopodidae, Psychodidae, Chironomidae

Introduction

In 1609 Galileo was the first human to point a telescope at the night sky and discover a huge number of stars never before seen (Galilei 1610). Initially he wanted to describe all those in the constellation Orion but was overwhelmed by what he estimated to be 500 new stars. He restricted himself to describing the 83 stars in the belt and sword region. And so science has progressed step-by-step, using new tools and approaches to discover new horizons of complexity and unsuspected diversity. Like Galileo, in our study 59 expert fly systematists focused their attention on the diversity of flies (Diptera) at a single cloud forest site in the highlands of Costa Rica. The project presents the first comprehensive, species-level inventory of Diptera at any mainland tropical site in the world. This paper reports unprecedented high numbers of fly species, suggesting, as did Galileo's magnified view of the heavens for stars, that there is a huge, unreported diversity of species of Diptera, at least in the Neotropical Region and probably also in other tropical areas of the planet.

The most completely described insect faunas are those of the northern temperate region. In chapter 3 of the *Origin of Species*, Darwin (1859) recognized, as did many other biologists at the time, that more species were in the tropics than in northern regions. "How many more?" has been the question ever since (Berenbaum 2009). Four orders of insects presently make up more than half of all named Animalia: the Coleoptera (beetles, 25.3%), Lepidoptera (moths and butterflies, 10.3%), Diptera (flies, 10.2%) and Hymenoptera (wasps, 7.6%) (Zhang 2011a, 2011b). A number of attempts have been made in the past few decades to estimate total numbers of species actually present. Erwin's (1982) seminal and provocative paper studied the number of beetle species on 19 individuals of a Panamanian rainforest tree (*Luehea seemannii* Triana & Planch.) and projected 30 million species of tropical

arthropods on Earth. Subsequently there have been a number of projections and conjectures as to the total number of arthropod or insect species, summarized by Berenbaum (2009), Erwin (2004) and Stork *et al.* (2015). These estimates have been based on data from one or more of the following approaches: host specificity, ratios of known to unnamed species, plant-herbivore ratios, higher taxonomic ratios, taxonomists' estimates, proportions of new species, body size in relation to the year of description, number of areas of endemism, rates of species description, and various mathematical models to reinterpret variable parameters (Amorim & Pires 1996; Basset *et al.* 1996; Costello *et al.* 2012, 2013; Gaston 1991; Groombridge & Jenkins 2002; Hamilton *et al.* 2010; Hammond 1992, 1995; Larsen *et al.* 2017; May 1990, 2000; Mora *et al.* 2011; Nielsen & Mound 2000; Raven & Yeates 2007; Stork 2018; Stork & Gaston 1990; Stork *et al.* 2015). Such estimates are also the basis for predicting extinction rates (Costello *et al.* 2013). These studies generally predict totals of 1.8 to 10 million insect species but they vary so widely that Caley *et al.* (2014) concluded that estimates of numbers of species via modelling are not converging—an indication that some or possibly all previous approaches are seriously flawed. Stork *et al.* (2015), however, argued that for Coleoptera there was congruence between 1) host specificity relationships and ratios, 2) ratios with other taxa and 3) the average body-size collected over time. They estimated the presence of 0.9–2.1 million species of beetles worldwide and used the ratio of known Coleoptera to other groups to suggest that there are 2.6–7.8 million species of insects on our planet.

Most previous efforts to determine the number of insect species in tropical locations have been swamped by several factors: (1) vastly overestimating the size of the area that could be adequately interpreted and resulting in too many specimens; (2) huge numbers of uncurated (unprepared) specimens (especially of the "difficult" groups); (3) poor organisation for sample processing and dissemination (including lack of care of fragile specimens); (4) a lack of systematists with the expertise to identify the resultant material; (5) constrained funding; (6) or usually, by a combination of these factors (Borkent & Brown 2015). Other more focused efforts, such as the sampling undertaken by Erwin (2004), Erwin & Geraci (2009) and Erwin *et al.* (2005), have projected global diversity levels based on relatively limited samples such as those taken by fogging small patches of trees in the Neotropical Region. Previously the most complete survey of a megadiverse order of insects in the tropics was that by Hammond (1990), reporting 3,488 species of Coleoptera from a 500 ha area in Sulawesi.

For our study, we chose a novel and direct approach based on a comprehensive but limited sampling protocol. Our method was to strongly constrain the sampling and processing of material by sampling a four-hectare plot of cloud forest at Zurquí de Moravia, San José Province, Costa Rica (hereafter referred to only as Zurquí) for one year. Identification of the Diptera from a single Malaise trap was considered a minimum contribution by participating systematists. However, in response to initial invitations, all the samples from more extensive collecting methods at this site (Borkent & Brown 2015; Fig. 2) were studied by all coauthors, other than for the hyperabundant Phoridae. A key aspect of the project was to have the material fully curated (including sophisticated slide mounting for several families), allowing participating specialists to promptly study the material.

Our paramount goal was to determine the number of dipteran species present at our primary site at Zurquí and to compare this to limited sampling from two other Costa Rican locations at a similar elevation. Our commitment to these goals pushed us towards the challenging route of actually studying all the species collected at Zurquí (including those families of flies previously deemed as impossible) by utilizing the skills of six technicians to fully sort and prepare material, and dividing the material among coauthors for determination to the species level. As expected, the diversity was strikingly high in this relatively small patch of cloud forest.

Borkent & Brown (2015) provide a synopsis of why Diptera are particularly suited to biodiversity studies. In summary, Diptera as a group are remarkably diverse at the species level, currently with 159,051 named species (Table 2) and vastly more unnamed, especially in tropical regions. In conjunction with this great diversity, Diptera display an extraordinary range of morphological divergence and ecological adaptations and are found in virtually every conceivable terrestrial and aquatic (limnic, brackish, saline, even some marine) microhabitat. They occur abundantly and display great species diversity worldwide (Marshall 2012; Pape *et al.* 2009; Yeates and Wiegmann 2005). As such, Diptera have a huge ecological repertoire that makes Diptera inventories excellent candidates for estimating ecological heterogeneity at a more detailed and diversified level than other taxon-specific inventories (Kitching *et al.* 2005; Smith & Mayfield 2015).

The present project was built on an earlier collaborative venture in which experts for each family of Diptera in Central America cooperated to produce two large volumes of the *Manual of Central American Diptera* (MCAD—Brown *et al.* 2009, 2011). Authors described, family-by-family, the genera present in Central America, producing syntheses of the fauna of each family and well-illustrated keys to their genera and discussing the known and

projected number of species in each genus. These manuals provided an excellent basis for pursuing the next level of systematic sophistication—that of the species level at a given Central American location.

Finally, there is currently a community of cooperating Diptera systematists able to work as a team to interpret each family of Diptera at our study site (Table 1). This complete coverage is unusual within the entomological community. In spite of the lamentable failure of our society to recruit a sufficient number of systematists to broadly interpret the biodiversity that is rapidly disappearing from our planet, the Diptera community still has a combination of employed, retired, and independent systematists who are actively enthused about pursuing species-level taxonomy in their respective families.

TABLE 1. Families of Diptera (n= 76) found at Zurquí, Tapantí and/or Las Alturas indicating taxonomic specialists, numbers of specimens extracted or selected, and numbers of species sampled at each site. Families studied at all three sites are shown in bold (Dolichopodidae only partly studied at Tapantí and Las Alturas). Extracted indicates all specimens curated; selected indicates specimens selected as distinctive morphospecies from a given sample.

* Empidoidea (other than Dolichopodidae) were identified but considered duplicates of morphospecies of Empididae or Hybotidae, females which could not be determined to morphospecies, or damaged specimens; they were all Empididae or Hybotidae but were not distinguished in the database.

** Phoridae were treated differently from all other families. Because of their overwhelming numbers, distinctive morphospecies were picked from the separated specimens (as a family) and curated without counting the numerous duplicates left uncurated in alcohol.

*** Sciaroidea *incertae sedis* refers to a member of the *Ohakunea* group recognized in Brown *et al.* (2009).

**** Syrphidae identified to species were completed by F.C. Thompson and M.A. Zumbado; additional generic identifications by M. Hauser.

Family	Coauthors	all extracted or morphospecies selected	total # specimens from all sites	# species Zurquí	# species Tapantí	# species Las Alturas
Agromyzidae	Stephanie Boucher	extracted	976	117	52	39
Anisopodidae	Dalton de Souza Amorim	extracted	605	27	15	1
Anthomyiidae	Verner Michelsen	extracted	83	7		
Anthomyzidae	Kevin Barber	extracted	60	2	0	0
Asilidae	Eric Fisher	extracted	148	20	0	0
Asteiidae	John Swann	extracted	1	1		
Athericidae	Norman Woodley	extracted	40	0	1	1
Aulacigastridae	Alessandra Rung	extracted	11	2	1	0
Bibionidae	Dalton de Souza Amorim	extracted	472	12	8	9
Bombyliidae	Brian V. Brown	extracted	1	1	0	0
Brachystomatidae	Jeffrey M. Cumming, Bradley J. Sinclair	selected	31	4		
Calliphoridae	Terry Whitworth	extracted	535	14		
Cecidomyiidae	Mathias Jaschhof	selected	3820	800		
Ceratopogonidae	Art Borkent	selected	4278	200	130	17

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TABLE 1. (Continued)

Family	Coauthors	all extracted or morphospecies selected	total # specimens from all sites	# species Zurquí	# species Tapantí	# species Las Alturas
Chamaemyiidae	Stephen D. Gaimari	extracted	36	1	1	0
Chironomidae	J.H. Epler	selected	2024	138		
Chloropidae	Terry Wheeler	selected	1749	88	34	29
Clusiidae	Owen Lonsdale	extracted	181	14	4	10
Conopidae	Jeffrey H. Skevington	extracted	93	9	5	7
Corethrellidae	Art Borkent	selected	283	7	7	0
Ctenostylidae	Valery Korneyev	extracted	1	1		
Culicidae	Thomas J. Zavortink	extracted	54	13	1	4
Diadocidiidae	Peter H. Kerr	selected	1	1	0	0
Diastatidae	Wayne Mathis	extracted	12	1	0	0
Ditomyiidae	Peter H. Kerr	selected	109	8	0	8
Dixidae	Art Borkent	extracted	65	2	0	0
Dolichopodidae	Marc Pollet (coordinator), Daniel Bickel, Scott E. Brooks, Renato Capellari, Stefan Naglis, Justin Runyon	selected	8346	178	35	10
Drosophilidae	David A. Grimaldi	selected	5098	219		
Empididae	Jeffrey M. Cumming, Bradley J. Sinclair	selected	376	35		
Empidoidea *	Jeffrey M. Cumming, Bradley J. Sinclair	selected	1686			
Ephydriidae	Daniel N.R. Costa, Wayne N. Mathis	extracted	2149	37	24	13
Fanniidae	Jade Savage	extracted	139	12		
Heleomyzidae	Norman Woodley	extracted	14	2	0	1
Hippoboscidae	Carl W. Dick	extracted	1	1	0	0
Hybotidae	Jeffrey M. Cumming, Bradley J. Sinclair	selected	1051	58		
Inbiomyiidae	Brian V. Brown	extracted	28	1	1	1
Keroplastidae	Peter H. Kerr	selected	12	9	1	2

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TABLE 1. (Continued)

Family	Coauthors	all extracted or morphospecies selected	total # specimens from all sites	# species Zurquí	# species Tapantí	# species Las Alturas
Lauxaniidae	Stephen D. Gaimari	selected	1031	116	28	32
Lonchaeidae	Allen L. Norrbom, Cheslavo Korytkowski	extracted	36	11	1	3
Lygistorrhinidae	Peter H. Kerr	extracted	3	1	0	0
Micropezidae	Stephen A. Marshall	extracted	165	12	6	10
Milichiidae	John Swann	extracted	1109	24	7	4
Muscidae	Jade Savage	selected	2157	120	36	15
Mycetophilidae	Peter H. Kerr	selected	2202	267	24	30
Neriidae	Alessandre Pereira-Colavite	extracted	20	1	1	2
Odiniidae	Stephen D. Gaimari	extracted	1	1		
Oestridae	Thomas Pape	extracted	1	1	0	0
Periscelididae	Alessandra Rung	extracted	307	15	15	4
Phoridae **	Brian V. Brown, John Hash, Giar-Ann Kung, Maria Wong	selected	453	407		
Piophilidae	Sabrina Rochefort, Stephen D. Gaimari	extracted	2	2	0	0
Pipunculidae	Jeffrey H. Skevington	extracted	183	70	14	26
Pseudopomyzidae	Tiffany Yau	extracted	376	6	2	4
Psilidae	John Swann	extracted	4	0	0	1
Psychodidae	Greg Curler, Gunnar Mikalsen Kvifte, Sergio Ibáñez-Bernal	selected	1780	171	30	25
Pyrgotidae	Valery Korneyev	extracted	2	1	1	0
Rhagionidae	Norman Woodley	extracted	364	13	12	4
Rhinophoridae	Thomas Pape	extracted	4	3		
Richardiidae	Valery Korneyev	extracted	27	6	4	1
Sarcophagidae	Thomas Pape	extracted	977	20		
Scathophagidae	Verner Michelsen	extracted	2	1		
Scatopsidae	Dalton de Souza Amorim	extracted	603	22	4	1
Sciaridae	Heikki Hippa, Pekka Vilkkamaa	selected	2007	204		

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TABLE 1. (Continued)

Family	Coauthors	all extracted or morphospecies selected	total # specimens from all sites	# species Zurquí	# species Tapantí	# species Las Alturas
Sciaroidea <i>incertae sedis</i>***	Peter H. Kerr	extracted	1	0	1	0
Sepsidae	Vera C. Silva	extracted	322	14	2	6
Simuliidae	Peter H. Adler	extracted	1137	10	5	5
Sphaeroceridae	Stephen A. Marshall, Steven Paiero, Tiffany Yau	selected	4308	77		
Stratiomyidae	Norman Woodley	extracted	291	36	19	19
Streblidae	Carl W. Dick	extracted	68	8		
Syrphidae****	Manuel A. Zumbado, Martin Hauser	extracted	417	93		
Tabanidae	John Burger	extracted	541	8	7	4
Tachinidae	D. Monty Wood, Manuel Zumbado, Z.L. Burington, John O. Stireman III	extracted	1276	286	58	52
Tanypezidae	Owen Lonsdale	extracted	37	2	1	1
Tephritidae	Allen L. Norrbom	extracted	75	21	4	3
Therevidae	Stephen D. Gaimari	extracted	4	0	0	3
Tipulidae	Jon Gelhaus	selected	2944	225		
Ulidiidae	Valery Korneyev, Elena P. Kameneva	extracted	89	13	6	3
Xylomyidae	Norman Woodley	extracted	6	1	1	2
Xylophagidae	Norman Woodley	extracted	4	1	0	1
Total			59905	4332	609	413

TABLE 2. Families of Diptera (n=73) found at Zurquí, comparing numbers of both described and undescribed species collected at Zurquí with named species from Central America (including tropical Mexico), Colombia, the Neotropical Region and the world. Numbers of Central American Diptera from Brown *et al.* (2009, 2011) with additions for a few families from coauthors. Colombian species recorded by Wolff *et al.* (2016), with additions and modifications as noted in text. Neotropical species from Pape & Thompson (2013) with some modifications by coauthors and Culicidae by R. Harbach (pers. comm.). World species from Pape & Thompson (2013) with modifications to Aulacigastridae (Rung & Mathis 2011), Brachystomatidae, Empididae and Hybotidae (Courtney *et al.* 2017), Cecidomyiidae (Gagné & Jaschhof 2014), Ceratopogonidae (Borkent 2016), Corethrellidae (Borkent 2014), Clusiidae (compiled by O. Lonsdale), Culicidae (Harbach 2016), Drosophilidae (Bächli 2017), Milichiidae (Swann 2016), Periscelididae (compiled by A. Rung), Pseudopomyzidae (compiled by T. Yau), Tephritidae (compiled by A.L. Norrbom), Sciaridae (Amorim & Schühli 2017), Simuliidae (Adler & Crosskey 2012), Sphaeroceridae (Bergeron *et al.* 2015; Kits 2015; Kits & Marshall 2013; Luk & Marshall 2014; Marshall 2013, 2014; Marshall *et al.* 2011; Papp 2013), Tachinidae (O'Hara 2013), Tipulidae (Oosterbroek 2017), Ulidiidae (compiled by V. Korneyev).

Family	total # species Zurquí	# named species Central America	# named species Colombia	# named species Neotropics	# named species World
Cecidomyiidae	800	250	44	567	6203
Phoridae	407	500	225	1645	4105
Tachinidae	286	210	154	2730	8500
Mycetophilidae	267	95	12	1065	4164
Tipulidae	225	629	131	3452	15457
Drosophilidae	219	300	176	884	4315
Sciaridae	204	130	16	294	2500
Ceratopogonidae	200	365	235	1132	6267
Dolichopodidae	178	400	29	1207	7236
Psychodidae	171	274	199	997	2958
Chironomidae	138	115	30	1069	7054
Muscidae	120	150	108	898	5210
Agromyzidae	117	85	74	464	2977
Lauxaniidae	116	178	36	370	1895
Syrphidae	93	473	312	1623	6016
Chloropidae	88	125	46	439	2880
Sphaeroceridae	77	140	25	474	1880
Pipunculidae	70	90	10	253	1420
Hybotidae	58	97	19	318	1971
Ephydriidae	37	280	53	395	1992
Stratiomyidae	36	330	86	934	2666
Empididae	35	50	8	514	3142
Anisopodidae	27	11	2	69	159
Milichiidae	24	56	6	124	364
Scatopsidae	22	70	2	67	390
Tephritidae	21	274	93	950	4911
Asilidae	20	393	72	1503	7479
Sarcophagidae	20	300	102	876	3094
Periscelididae	15	9	3	55	127

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TABLE 2. (Continued)

Family	total # species Zurquí	# named species Central America	# named species Colombia	# named species Neotropics	# named species World
Sepsidae	14	25	10	44	384
Calliphoridae	14	43	52	132	1522
Clusiidae	14	159	23	313	360
Culicidae	13	400	354	982	3550
Rhagionidae	13	70	3	103	711
Ulidiidae	13	143	28	29	875
Bibionidae	12	44	17	190	760
Fanniidae	12	12	32	85	359
Micropezidae	12	50	57	288	579
Lonchaeidae	11	29	44	95	504
Simuliidae	10	94	67	382	2132
Conopidae	9	57	16	209	783
Keroplastidae	9	35	5	196	945
Ditomyiidae	8	10	0	31	94
Streblidae	8	99	73	153	239
Tabanidae	8	186	255	1176	4406
Anthomyiidae	7	30	6	105	1927
Corethrellidae	7	41	9	77	104
Pseudopomyzidae	6	4	0	9	24
Richardiidae	6	15	19	167	175
Brachystomatidae	4	0	0	61	153
Rhinophoridae	3	12	1	20	174
Anthomyzidae	2	5	1	9	135
Aulacigastridae	2	2	1	37	55
Dixidae	2	13	1	29	186
Heleomyzidae	2	2	2	90	728
Piophilidae	2	4	2	11	82
Tanypezidae	2	8	9	19	21
Asteiidae	1	12	0	32	136
Bombyliidae	1	134	22	717	4946
Chamaemyiidae	1	9	0	54	350
Ctenostylidae	1	3	0	5	10
Diadocidiidae	1	3	0	7	34
Diastatidae	1	1	0	4	48
Hippoboscidae	1	27	18	32	213
Inbiomyiidae	1	5	0	11	11
Lygistorrhinidae	1	3	0	7	33
Neriidae	1	20	17	40	112
Odiniidae	1	11	0	25	64

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TABLE 2. (Continued)

Family	total # species Zurquí	# named species Central America	# named species Colombia	# named species Neotropics	# named species World
Oestridae	1	21	3	38	190
Pyrgotidae	1	11	1	55	350
Scathophagidae	1	3	1	5	414
Xylomyidae	1	10	1	9	134
Xylophagidae	1	2	1	12	136
Other families	0	191	64	1036	12571
Total	4332	8437	3523	32499	159051

Materials and Methods

The family classification follows that of Brown *et al.* (2009, 2011), other than the Empididae *sens. lat.* which are here treated as three families, namely Empididae, Brachystomatidae and Hybotidae, following Sinclair & Cumming (2006). Borkent & Brown (2015) provided a detailed description of the primary methods used for the ZADBI project (Zurquí All Diptera Biodiversity Inventory), summarized as follows.

The primary study site was limited to a 150 x 266 m patch of cloud forest at Zurquí, Costa Rica (10.047°N, 84.008°W), at about 1,600 meters elevation (Fig. 1A). It was sampled continuously with two Malaise traps for slightly more than one year (Sept. 12, 2012–Oct. 18, 2013) with Malaise trap #1 placed near a forest-edge and Malaise trap #2 inside of the forest, near a small permanent creek. In addition, we undertook concomitant sampling with a variety of trapping methods for three full days every month (these traps were picked up on the fourth day) including light traps (CDC, bucket traps, UV light over pan of soapy water), emergence traps over a wide array of terrestrial and aquatic substrates, baiting with various attractants (fruit, carrion, human and pig dung), yellow pan traps, a flight-intercept trap, two other Malaise traps with ethanol as preservative, a dry Malaise trap using potassium cyanide as the killing agent (to capture larger specimens dry) and a canopy Malaise trap. An intensive "Diptera Blitz", with 18 coauthors collecting on-site August 5–9, 2013, provided diverse additional samples used in the inventory. Although demarking an area of about 4 hectares (0.04 square kilometers), in practice nearly all the supplemental collecting with other trapping methods was restricted to habitat adjacent to the trails, along the permanent stream or in the open meadow and area near the cabins (Fig. 1A).

Two other Costa Rican sites, at Tapantí National Park, Cartago Province (9.720°N, 83.774°W, 1,600 m) and Las Alturas, Puntarenas Province (8.951°N, 82.834°W, 1,540 m), 40 and 180 km southeast from Zurquí (Fig. 1B), respectively, were each sampled for a similar length of time with a single Malaise trap placed on the forest edge to allow for limited beta-diversity assessments for a number of families (those which were fully extracted or subsampled at all three sites) (Table 1). Tapantí National Park was sampled from Oct. 28, 2012–Oct. 13, 2013 and Las Alturas from Oct. 13, 2012–Oct. 13, 2013. Malaise traps #1 and #2 at Zurquí and those at the other two sites were emptied weekly.

Nearly all specimens in most families were fully curated (e.g., pinned or slide mounted) with exceptions for a few coauthors who requested their material in ethanol. Those in superabundant families (often with many thousands of specimens), were subsampled by the technicians (Table 1), with any specimen perceived to be morphologically different within a sample selected and prepared (Table 1). The Cecidomyiidae, however, were studied and subsampled by M. Jaschhof and the Phoridae by B.V. Brown, J. Hash, G. Kung, and M. Wong. In addition, a few coauthors studied residue material retained in ethanol, considered by the technicians to be redundant morphospecies.

An international group of 59 expert systematists (the coauthors) morphologically identified specimens of all 76 dipteran families present at one or more of the three sites (Table 1) to species or morphospecies. Six local technicians sampled and prepared material to the highest curatorial standards, ensuring that the energy and time of each coauthor were focused on species identification. During the course of this study, the project was supported by two sequential project managers who helped direct material to coauthors.

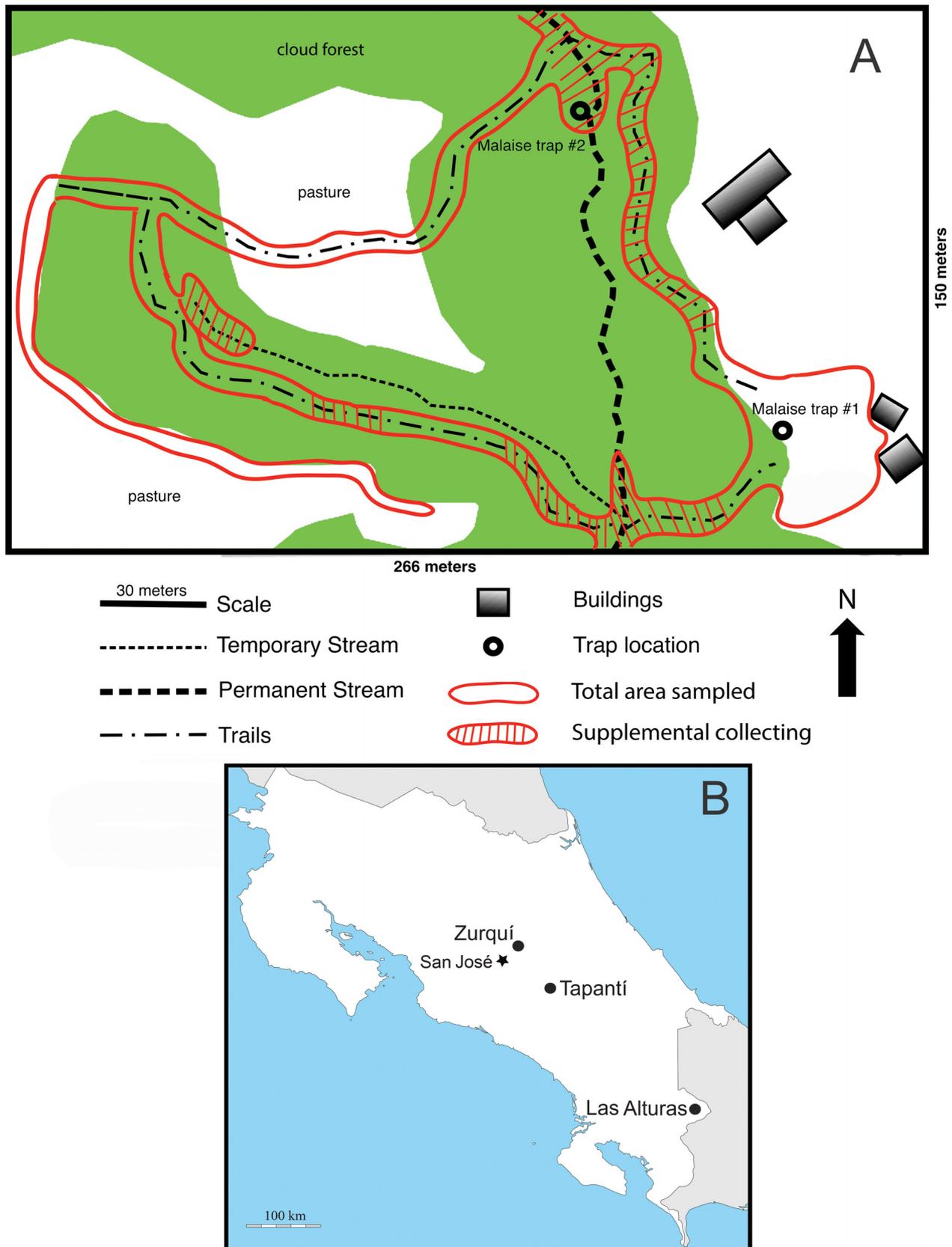


FIGURE 1. (A) Map of study site at Zurquí de Moravia, Costa Rica, with general features and primary collecting localities indicated (modified from Borkent & Brown 2015). Supplemental collecting indicating those areas where other collecting generally took place for three days of each month. (B) Map of Costa Rica showing location of the three collecting sites. San José indicated for reference.

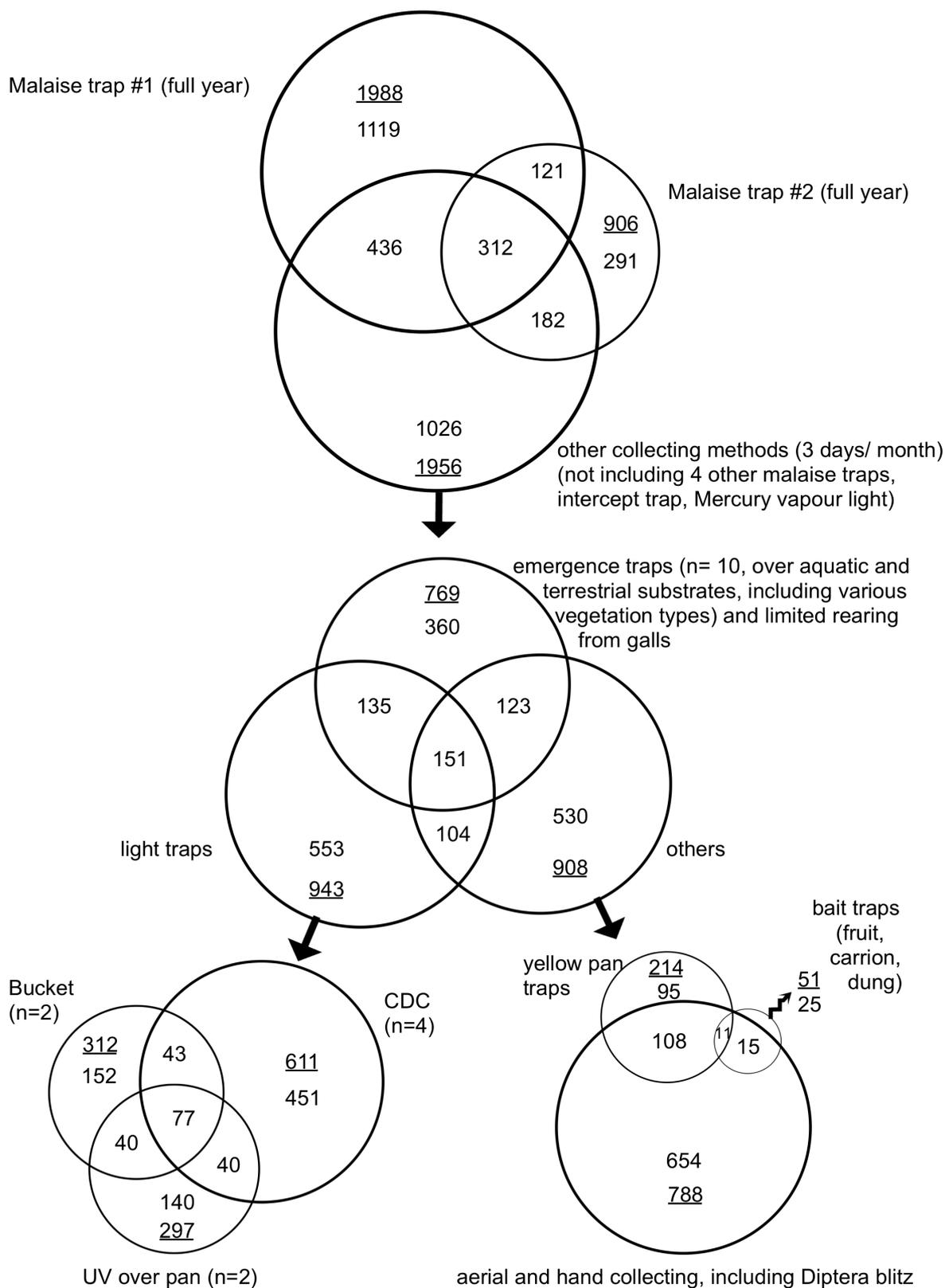


FIGURE 2. Venn diagrams indicating number of species at Zurquí shared by each of Malaise traps #1 and #2 and all other methods combined, with the latter further separated into main elements (excluding Phoridae, other Malaise traps, flight-intercept trap, Mercury vapour light; total of 3,487 species considered here). Total number for a given method underlined. Those not underlined are either unique or overlapping within that group of collecting methods.

A substantial proportion of species in various families and/or genera could not be named due to a lack of comprehensive revisions for these taxa. For many taxa, it was uncertain whether they represented undescribed species or were species that are already named but are inadequately diagnosed. These taxa were simply recognized as morphospecies and provided with a numerically based code (ZUR-1, ZUR-2, etc.). Specimens are currently housed either at the Los Angeles County Museum or retained by coauthors for further study (temporarily or, in the case of duplicates permanently). Many of these (and all holotypes) are to be returned to the Museo Nacional de Costa Rica which recently assumed responsibility for the collection previously housed at the Instituto Nacional de Biodiversidad in Costa Rica.

The total named and unnamed fauna at Zurquí was compared to the named fauna of Central America, Colombia and larger areas. The fauna of Costa Rica was not compared because the number of named species is not recorded for most families of Diptera.

The entire data set is stored as a MySQL database at www.phorid.net, with a copy to be deposited in the online depository DRYAD (<http://datadryad.org>).

Results

Our sampling from all three sites, with 717 sampling events, resulted in the curation of a total of 62,410 specimens, with 2,505 of these damaged, lost or directed to the wrong coauthor, leaving 59,905 specimens to be studied (Table 1). Of these, 74 of the 76 families were completely identified (some only from Zurquí; Table 1), leaving only the abundant, diverse and challenging Drosophilidae and Tipulidae with 29% and 62% identified, respectively. Phoridae, with only 453 curated specimens (Table 1), requires comment. Unlike other abundant families that were selected from ethanol by our technicians on a sample-by-sample basis, Phoridae were selected with a goal of cumulatively picking out additional species as samples were examined, leaving the remaining material in ethanol (and not curated). It was estimated, based on counts from two representative samples that more than 50,000 phorids were examined.

A total of 52,947 specimens was available for study from Zurquí, with its multiple collecting methods, and 4,434 and 2,524 specimens of select families from Tapantí and Las Alturas, respectively. Out of 107 families of Diptera known from Costa Rica (Brown *et al.* 2009, 2011; including four families of Empidoidea recognized here), 76 families were collected at least at one of the three sites (Table 1), with 73 families recorded at Zurquí. The three additional families not present at Zurquí were Athericidae (1 species, 36 specimens at Tapantí and 4 specimens at Las Alturas), Psilidae (1 species, 4 specimens at Las Alturas) and Therevidae (3 species, 4 specimens at Las Alturas). A single specimen of a Sciaroidea *incertae sedis* was present at Tapantí.

Diversity at Zurquí. A remarkable total of 4,332 species in 73 families was recorded at Zurquí, revealing a previously undocumented level of diversity within the Diptera. Species accumulation curves based on Chao 1 and rarefaction of 49 fully extracted families (Table 1) from Malaise traps #1 and #2 indicate 1.8 times more species are actually present at Zurquí, giving a total for Zurquí of more than 8,000 species (Brown *et al.*, unpublished data).

Further evidence that our total numbers are underestimates comes from a few taxa in which the technicians' subsampling protocol was checked by specialists. In each case, further species were found in the "residues" from which subsamples had been previously extracted. For example, specimens of Lauxaniidae had been extracted and curated by our technicians and the remaining supposed supplemental specimens relegated to vials, as for other abundant families. These ethanol specimens were subsequently re-examined by one of us (S.D. Gaimari) and a further 24 species recognized (and then included in the database). Many of these had subtle differences from the previously pinned material, indicating that for this family, at least, the selection process was only 79% successful. This particular case suggests that our protocol overlooked at least some species but of an unknown percentage of the total Diptera, that probably would have been significantly reduced if taxonomic experts were selecting specimens from the samples. However, even with expert surveillance, one of us (M. Jaschhof), responsible for curating Cecidomyiidae to be slide mounted, estimated that at least 10% of the species were missed, most of which would have required slide mounting to determine further subtle differences between species (i.e., otherwise appearing similar when in alcohol). It was apparent that hyperabundant families like Cecidomyiidae and Phoridae need extraordinary curatorial efforts in inventories like ours (see Discussion).

We consider virtually all our species as native and endemic to at least the New World with few exceptions. Some were secondarily widespread species associated with humans or domestic animals (cattle were in the meadow at our site). The two species of Piophilidae recorded, *Piophila casei* (Linnaeus) and *Stearibia nigriceps* Meigen, are virtually cosmopolitan and associated with dried protein of various sorts (Rocheftort *et al.* 2015). Other introduced species include the cosmopolitan psychodid *Psychoda alternata* Say, associated with anthropized environments and human dwellings, the phorid *Megaselia scalaris* (Loew) and two muscids, *Stomoxys calcitrans* (Linnaeus), an Old World biting pest of cattle and humans (larvae breed in manure and rotting vegetation) and *Musca domestica* Linnaeus (another originally Old World but now cosmopolitan fly). In addition, some species may be naturally more broadly distributed, such as seven species of fungus-feeding Cecidomyiidae and two species in the leaf-mining family Agromyzidae: *Cerodontha dorsalis* (Loew) and *Nemorimyza posticata* (Meigen) which are known otherwise in the New World and Palaearctic Region.

By far the most diverse family at Zurquí was the Cecidomyiidae with 800 species (Table 2). This abundant and cosmopolitan family, commonly known as gall midges, actually includes many taxa that are not gall inducers (or other plant feeders) but are either free-living fungal feeders (including all basal lineages and ¼ of all described species), inquilines, predators or even parasitoids. Gall formers are restricted to groups within the subfamily Cecidomyiinae and historically taxonomists of this group have collected galls or otherwise affected plants and described the reared adults, associated larvae and pupae and gall morphology. As such, many described taxa are most easily identified on the basis of their host plant and are largely restricted to those galls detectable by human sight (e.g., Dalbem & Mendonca 2006; Gagné 1989, 1994). Collecting visible cecidomyiid galls over several years has revealed only 43 different galls on plants at Zurquí (P. Hanson, pers. comm.), emphasizing the importance of collecting adult cecidomyiids using more general techniques to discover their true diversity. This striking diversity is discussed further below.

Other families were also outstandingly diverse. The second greatest number of species was in the family Phoridae, with 407 species recognized. This family has long been known to be extremely diverse, with species that are parasitoids, parasites, predators, fungivores, scavengers (some highly specialized), herbivores, and a few well-known generalists (Brown 2005). They are found worldwide, although they are most diverse in humid (not necessarily tropical) localities.

Tachinidae were the third most diverse family with 286 species. All members of this family are obligate endoparasitoids of insects, especially plant-feeding larvae of Lepidoptera, Coleoptera, and Hymenoptera. There are more than 8,500 species described worldwide, and they are abundant in nearly all terrestrial habitats. More than 60% of recorded Zurquí species were collected only once or twice and many of these likely represent host-specialized cryptic species complexes (Smith *et al.* 2007), indicating that the current count from Zurquí is likely a substantial underestimate of the total fauna. The most species rich genera from Zurquí, including *Phytomyptera* Rondani (18 spp.), *Siphona* Meigen (15 spp.), and *Chaetostigmoptera* Townsend (16 spp.), are typically less than 5 mm in body length.

The fourth most diverse was the Mycetophilidae with 267 species. As their common name “fungus gnats” suggests, species are dependent on fungi for their development, either within mushroom fruiting bodies or in decomposing wood and humus where they feed on fungal mycelia. In most habitats where they occur, they are an especially abundant, diverse, and easily captured component of the insect fauna. At Zurquí, a little over half of the mycetophilid diversity (136 species) was found within a single cosmopolitan genus, *Mycetophila* Meigen. This is quite different from north temperate areas, where *Mycetophila* typically makes up a much smaller proportion of the known family diversity.

Tipulidae were the fifth most diverse family of flies at Zurquí, with 225 species in 33 genera. This is nearly twice the recorded species for Costa Rica (presently 123 species), indicating how poorly known the species are for this country. Tipulids, also commonly known as crane flies, develop in a wide variety of habitats, from streams and rivers, seeps, phytotelmata to wet to drier earth, leaf litter, rotting wood, fungi and even leaves of terrestrial plants, all found at the Zurquí site. The genus *Dicranomyia* Stephens with 29 species and allies (*Geranomyia* Haliday, 23 spp., *Neolimonia* Alexander, 2 spp. and *Rhipidia* Meigen, 17 spp.) in the Limoniini comprise 31% of the species at Zurquí. Other species rich genera include *Teucholabis* Osten Sacken (18 spp.) and *Molophilus* Curtis (15 spp.), *Gonomyia* Meigen (12 spp.), *Erioptera* Meigen (11 spp.) and *Atarba* Osten Sacken (12 spp.). The genus *Tipula* Linnaeus, including some of the larger species of crane flies, includes 10 species.

The sixth most diverse was the Drosophilidae, represented by 219 species. The specimens were incompletely

identified and certainly additional species are present in the curated material. The genera of greatest diversity were *Drosophila* Fallén (59 species), *Zygothrica* Wiedemann (46 species), *Diathoneura* Duda (37 species), and *Cladochaeta* Coquillett (23 species), the last two being entirely New World (and almost entirely Neotropical) genera. Species of *Zygothrica* (e.g., Grimaldi 1987) aggregate at forest fungi, although some species breed in flowers, some species of *Diathoneura* are known to breed in flowers, and *Cladochaeta* are larval parasites of cercopid (spittlebug) nymphs (Grimaldi & Nguyen 1999). *Drosophila* breeding sites include living and fallen flowers, fruits, and fungi. The 219 species are equal to 73% of the described number of 300 drosophilid species for the entire country of Costa Rica (Bächli 2017).

Sciaridae were the seventh most species-rich family at Zurquí with 204 species and, like the Mycetophilidae, are associated with fungi and decaying organic matter. The 71 species of *Bradysia* Winnertz represented 35% of the Zurquí sciarid fauna.

Ceratopogonidae, with 200 species at Zurquí, were the eighth most species-rich family. Commonly known as biting midges or no-see-ums, this family includes 6,267 named species worldwide and about 365 in Central America (Table 2) (Borkent 2016). Four genera, *Atrichopogon* Kieffer, *Forcipomyia* Meigen, *Dasyhelea* Kieffer and *Culicoides* Latreille represent 59% of the world fauna and these represented 79% of the species at Zurquí. Immatures of these four genera are generally present in small water bodies, phytotelmata, mud, and wet vegetation (including mosses) and some are terrestrial (but then in moist habitats, such as under rotting bark). The remaining genera are fully aquatic. These habitats are abundant at Zurquí.

Other significantly diverse families with between 100 and 200 species (Table 2) were, in descending order, Dolichopodidae (n= 178), Psychodidae (n= 171), Chironomidae (n= 138), Muscidae (n= 120), Agromyzidae (n= 117), and Lauxaniidae (n= 116).

Comparisons of trapping methods at Zurquí. Different trapping methods (Borkent & Brown 2015) resulted in considerable differences in species sampled at Zurquí. Although no tests of statistical significance seem useful with these limited and biased techniques, several features were evident and likely valuable for future studies.

Here we present two views of our collecting efforts. First, to examine the relative contribution of different collecting methods for the inventory we compared the two continuously running Malaise traps with the other main collecting methods (Fig. 2). Because of their varied use during the three days per month of collecting, we excluded other supplemental Malaise traps, the flight-intercept trap and the mercury vapour light (with specimens collected by hand). Phoridae were also excluded because they were primarily extracted from Malaise trap #1 and would have biased comparisons between collecting methods. This left 3,487 (of the total 4,332) species to interpret. The 1,956 species from "other collecting methods" were further divided into various collecting techniques such as emergence traps (and limited rearing of galls from two plant species), various light traps (bucket, CDC, UV over pan) and "others", including yellow pan traps, bait traps (using various fruits, banana yeast, carrion (fish, chicken, liver) and dung (pig, human)) and those collected with aerial nets and hand collecting (including those collected by Diptera Blitz participants).

The forest-edge Malaise trap #1 (Fig. 1A) sampled a total of 1,988 species, representing 57% of the species considered here. Malaise trap #1 collected more than twice the total number of species as Malaise trap #2 in the forest at the stream (n= 906). "Other collecting methods" sampled 1,956 species, nearly equal in number to that of Malaise trap #1. Malaise trap #1 collected 1,119 unique species compared to only 291 unique species in Malaise trap #2, strongly supporting the known "edge effect" for Diptera of placing a Malaise trap on a forest margin to sample superior numbers of specimens and taxa (Ewers & Didham 2006; Matthews & Matthews 1970; Odum 1971; Ries *et al.* 2004). "Other collecting methods" sampled 1,026 unique species not collected by either Malaise trap, indicating their substantial importance in sampling Diptera for this inventory.

Of the 1,956 species collected by "other collecting methods", emergence traps over a variety of substrates sampled 769 species of which 360 were unique. A variety of light traps sampled 943 species, with 553 being unique and other methods, predominated by aerial and hand collecting, sampled 908 species, with 530 of these being unique.

Of the 943 species sampled with light traps, the single mercury vapour light was not included in the Venn diagram because specimens were selected by hand and sampling was likely highly biased (but still sampling 303 species). Of the remaining light traps, four CDC light traps collected 611 species of which 451 were unique, two bucket light traps collected 312 species of which 152 were unique and two UV lights over soapy water collected 297 species of which 140 were unique species.

The remaining collecting methods yielded a total of 908 species, including yellow pan traps, with 28 collecting events (1 event being equal to 3 days of sampling) and bait traps with 32 collecting events. These sampled only 95 and 25 unique species respectively (within this grouping of methods). Included in the aerial and hand collecting, which sampled 788 species of which 654 were unique, were 305 species from the Diptera Blitz of which 100 were unique to the project (including eight species of Streblidae, found only on bats), reflecting the specialized skills of systematists collecting their own groups in the field.

A second perspective on trapping methods simply considered all species collected by a given method (except Phoridae; total $n=3,943$) and compares each with the total sampled, regardless of biases in collecting methods (Table 3). The largest numbers of species were collected by Malaise trap #1, the combination of four Malaise traps and intercept trap, Malaise trap #2, aerial/hand collecting, emergence traps and CDC light traps. These also produced the largest numbers of unique species, indicating their importance for the collecting protocol used here. The unique species from each collecting method totalled 2,342 or 59% of all species collected.

Although a wide variety of trapping methods was used, it is likely that a number of species were not collected because they are strongly associated with specific microhabitats and/or are otherwise specialized. To sample such species requires either other types of collecting methods (McLean 2010) or specifically directed hand or aerial collecting.

It is important to remember that Malaise traps #1 and #2 were run continuously throughout the year, while other sampling methods, including supplemental Malaise traps, were used for only three days of every month. Comparisons between collecting methods always have a level of uncertainty because they attract and capture specimens in different ways (often for different periods of time) and so much depends on the behaviour of individual species, exact location and position of traps, as well as the microhabitats available. There will always be strong differences between collecting methods. Overall, our approach validates the use of a variety of methods to broadly inventory Diptera but with the recognition that Malaise traps, light traps and aerial/hand collecting sampled the bulk of the species present.

TABLE 3. Numbers of species at Zurquí sampled by each collecting method, numbers of species unique to that collecting method, and percentages of the total number of species that each represents (Phoridae not included, leaving a total of 3,943 species considered). MT refers to Malaise trap.

Collecting method	total	% of total	unique	% of total
MT #1	1988	50.4	872	22.1
MT #2	906	23.0	252	6.4
MT - all others + intercept	1282	32.5	305	7.7
MT - canopy	280	7.1	60	1.5
emergence traps + galls	769	19.5	185	4.7
Bucket light traps	312	7.9	37	0.9
CDC light traps	611	15.5	259	6.6
UV over pans of water	297	7.5	38	1.0
Mercury vapour light	302	7.7	50	1.3
yellow pan traps	214	5.4	21	0.5
bait traps	51	1.3	11	0.3
aerial/ hand collecting	779	19.8	243	6.2
from bats, bird	9	0.2	9	0.2

Comparative Diversity at Zurquí, Tapantí and Las Alturas. The diversity of 55 families of Diptera (those in bold in Table 1; plus Sciaroidea *incertae sedis*) from Zurquí, Tapantí and Las Alturas (Fig. 1B) was compared, suggesting several patterns. Each of Malaise trap #1 (Fig. 3A) and # 2 (Fig. 3B) and then all collecting methods (Fig. 3C) at Zurquí were compared with each of the Malaise traps at Tapantí and Las Alturas with Venn diagrams and Jaccard Index (JI) values (comparing total shared species/total species in two areas, here expressed as a percentage). In each of the three comparisons, the dipteran fauna at Zurquí was more similar to that at Tapantí than

to that of Las Alturas and the fauna at Tapantí more similar to that at Zurquí than to that of Las Alturas. These similarities generally correspond to or correlate negatively with the relative location and distances between the sites and suggest significant faunal turnover among the three sites. However, the correlation was not statistically significant in the dataset involving the Zurquí Malaise trap #1 (Fig. 3A; Pearson correlation coefficient -0.89, t-value -1.93, one-tailed p-value 0.07).

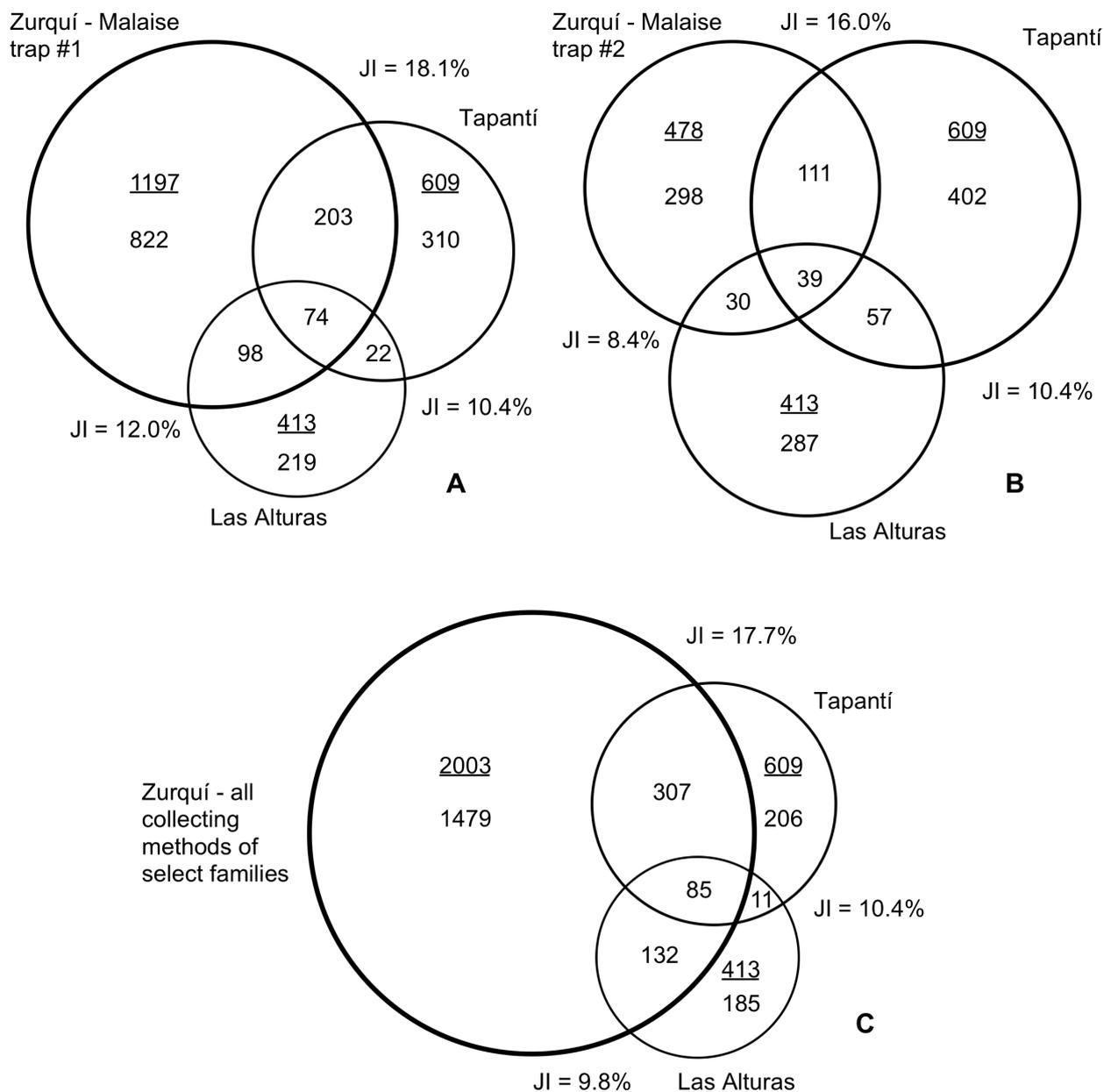


FIGURE 3. Venn diagrams indicating number of species shared by samples from Zurquí with species in a single Malaise trap at each of Tapantí and Las Alturas. Jaccard Index of similarity (JI) values are shown as percentages for each paired comparison. (A) Malaise trap # 1 at Zurquí. (B) Malaise trap # 2 at Zurquí. (C) All methods at Zurquí. Total number for a given site is underlined. Families studied at all three sites are shown in bold in Table 1.

The JI values between the Zurquí Malaise trap #1 and the other two sites (Fig. 3A) were similar to those between all collecting methods at Zurquí and the other two sites (Fig. 3C), even though supplemental sampling at Zurquí increased the number of species by 67% (i.e., from 1,197 to 2,003). The increased number of species sampled at Zurquí (Figs 3A, C) changed the similarity of this site to Tapantí from 18.1% to 17.7% and to Las Alturas from 12.0% to 9.8%. This indicates that expanding the collecting protocol at Zurquí did not markedly change the level of its similarity to the other two sites.

Even though JI values showed little change with the increase in number of species from Malaise #1 (Fig. 3A) to all collecting methods (Fig. 3C) from Zurquí, the numbers of species themselves show an increase in those shared with Tapantí (from 277 to 392) and with Las Alturas (from 172 to 217), indicating that the broader sampling at Zurquí increased the number of shared species by 41% and 26% respectively.

Comparison among the three sites for both Malaise traps #1 and #2 (Figs 3A, B) shows at least 51% of the species were unique at each site, also indicating marked species turnover among the three localities (e.g., 69% of the species from Malaise trap #1 were unique). Broadening the sampling protocol at Zurquí (Fig. 3C) produced a similar rate of 74% of species that were unique to this site but decreased the percentage of unique species at Tapantí and Las Alturas to 34% and 45% respectively. This suggests that increased sampling would decrease the apparent degree of species turnover.

These results may be biased by the difference in numbers of species (and specimens) sampled at each of the localities. For example, the decrease in levels of JI similarity when comparing Zurquí Malaise trap #1 (Fig. 3A) and Malaise trap #2 (Fig. 3B) to the other two sites may be influenced by the fewer species sampled by Malaise trap #2 ($n=478$) compared to Malaise trap #1 ($n=1,197$). So too, the Malaise trap at Las Alturas collected fewer species and specimens than those at the other sites.

Comparisons between the number of species at Zurquí and those named elsewhere. The 4,332 species of Diptera recovered at Zurquí after only one year of sampling is startling. However, we expect that should further comprehensive studies be undertaken, many tropical sites within at least much of the Neotropical Region will harbour equal if not higher numbers of species. The following analysis provides strong evidence that this will be the case.

Studies of two families that are relatively well known, the Culicidae and the Tabanidae, indicate high levels of diversity in the Neotropical Region. For example, there were 13 species of Culicidae found at Zurquí (with a limited sample size of 43 specimens), with two of these undescribed. The total fauna for Costa Rica is 243 species (including some undescribed species; compiled by T. Zavortink) and the entire Neotropical Region has at least 982 species (R. Harbach, pers. comm.). Tabanidae were represented by eight species at Zurquí (based on 52 specimens), while 146 are known from Costa Rica and 1,176 are present in the Neotropical Region (Table 2). If other families display similar patterns, where the Zurquí species are a small fraction of the total known from Costa Rica and the Neotropical Region, this suggests that the 4,332 species of Diptera at Zurquí are the proverbial tip of the iceberg and that a huge number of species remain to be discovered elsewhere.

To test this idea further, we compared the number of species (including unnamed morphospecies) at Zurquí with those named from Central America, Colombia, the Neotropical Region and the worldwide fauna (Table 2, Figs 4–7). We did not show Cecidomyiidae, with 800 species, in Figs 4–7 because they are so poorly known elsewhere that comparisons were extreme and therefore markedly affecting the scale of the graphs; the number of species of cecidomyiids at Zurquí are equal to 320% of those named from Central America, 141% of those from the Neotropical Region and 13% of those named worldwide.

It is an astonishing observation that the 4,332 Zurquí species collected in 0.04 square kilometers of cloud forest is equivalent to 51% of the 8,437 fly species named from Central America (Table 2), a diverse and complex region about 1,040,780 km² in size (our survey area was therefore 0.000004% of Central America). All 12 families with 100 or more species recorded at Zurquí, other than Dolichopodidae and Tipulidae, equalled more than half the named fauna of each of these families in Central America (Fig. 4). Six of the 12 had more species than are named for all of Central America. Twenty-two of the 45 families with eight or more species at Zurquí had at least 50% of the number of named species from Central America. Clearly, our limited but intense inventory at Zurquí indicates that the Diptera of Central America remain grossly understudied.

So too, a comparison with the described fauna of Colombia (Fig. 5) is striking. This country includes much of the northern end of the Andes and is known for its high diversity in many better known groups of organisms; it has 1,871 bird species (17% of world fauna), 456 species of mammals (8% of world fauna) and more than 750 amphibian species (12% of world fauna). There is little doubt that the insect fauna, although generally poorly studied, is also extremely diverse. The number of dipteran species at Zurquí surpasses that known for all of Colombia! Wolff *et al.* (2016) recorded 3,135 species for Colombia, not including eight families of Diptera (and missing Anthomyzidae). The number of named Colombian species of these nine families has been added to Table 2, as well as some minor changes to a few families (notably Ulidiidae - Kameneva *et al.* 2017 and Anthomyzidae -

Roháček & Barber 2009), giving a total of 3,523 recorded species. Certain families were particularly diverse at Zurquí when compared to the named species of Colombia. Of those 45 families at Zurquí with eight or more species present, 25 had more species inventoried than known for all of Colombia and 16 of these had more than three times that recorded from Colombia. The Cecidomyiidae and Mycetophilidae were especially striking, with 800 and 267 species known from Zurquí, respectively, and only 44 and 12 species known from Colombia, respectively. Clearly, the Colombian species of these and most other families are extremely poorly known. Similarly, Delgado Puchi (2003) listed only 59 families and 1,519 species of Diptera from Venezuela, so that the number of Diptera at Zurquí (named and unnamed) is 2.9 times the known species of Venezuela. Although he estimated an additional 336 undescribed species for that country, our inventory strongly suggests that the number of unnamed Diptera of Venezuela is certainly much higher.

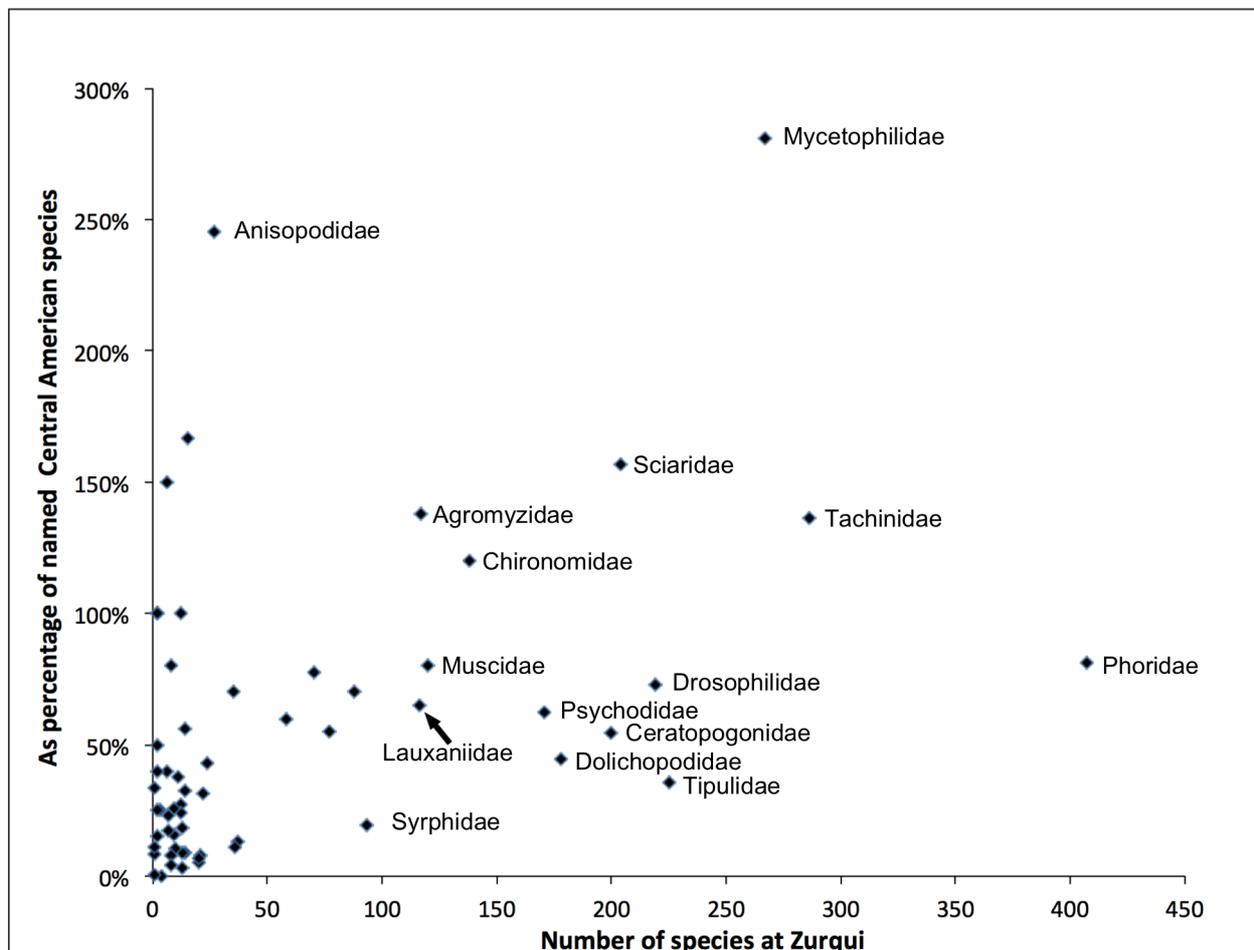


FIGURE 4. Relationship between number of all species occurring at Zurquí (named and unnamed) and all named species from Central America (not including Cecidomyiidae, with 800 species). Numbers of species from Brown *et al.* (2009, 2011), with additions for a few families from coauthors.

On a broader scale, the total number of species at Zurquí is equivalent to 14% of all those recorded for the entire Neotropical Region (Table 2) and 19% of the 22,229 known from the Nearctic Region (Pape & Thompson 2013). Of 25 families with 22 or more species from Zurquí, 20 had at least 10% of the named Neotropical species (Fig. 6). These comparisons indicate with certainty that the named species of the Neotropical Region are also severely underestimated.

Comparison of the Diptera of Zurquí and the total world fauna is similarly striking (Table 2, Fig. 7), with Zurquí Diptera equivalent to 2.7% of the named world species. Eleven families with 20 or more species at Zurquí were equivalent to 5–17% of named world species and 45 of the 73 families at Zurquí had $\geq 2\%$ of the named world fauna.

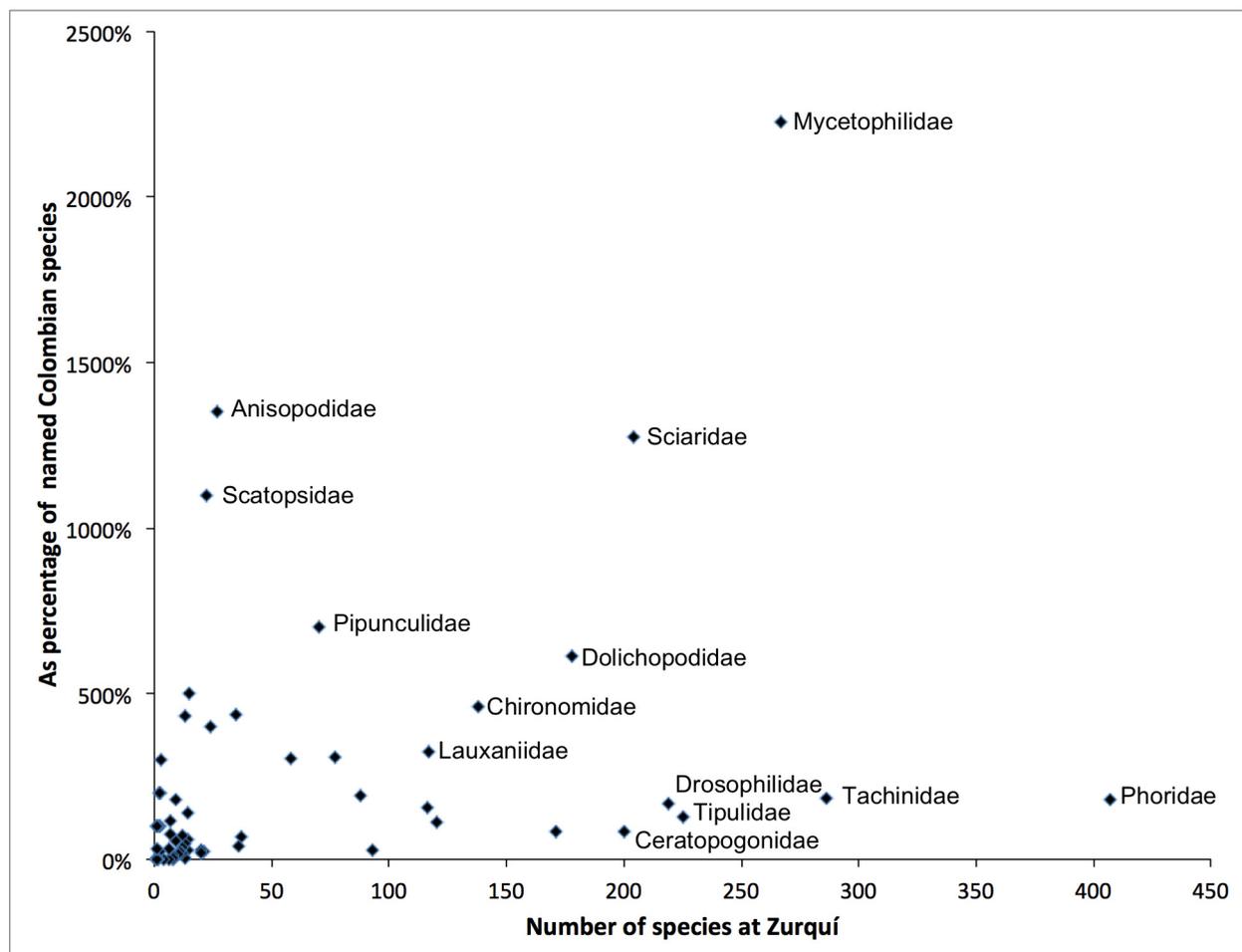


FIGURE 5. Relationship between number of all species occurring at Zurquí (named and unnamed) and all named species from Colombia (not including Cecidomyiidae, with 800 species). Numbers of species from Wolff *et al.* (2016) with additions and minor modifications noted in text.

The high number of species of Cecidomyiidae discovered at Zurquí requires special mention. Unfortunately, this family has not been systematically inventoried anywhere else in the world and represents the proportionally most poorly described family of Diptera. Although the subfamily Cecidomyiinae, including those taxa that form galls, is so poorly known, Brown (2009) provided a projection for this subfamily in Costa Rica of 18,000 species based on R. Gagné's suggestion that there are two species for every species of vascular plant (but see Bourg & Hanson (2014) for varying levels of host specificity). This estimation disregarded the predators among the Cecidomyiinae, which our project showed to contribute more than one-fourth to the species total in this subfamily. Also, Brown's (2009) estimate (based on the suggestion by M. Jaschhof) of 600 fungivorous Cecidomyiidae in Central America was certainly much too conservative, considering the fact that 314 fungivorous species were discovered at Zurquí (39% of all Cecidomyiidae found).

Other than the recent DNA barcoding study by Hebert *et al.* (2016), there have been no inventories using trap-collected material to estimate all-cecidomyiid diversity. However, it has long been recognized that Cecidomyiidae are the most abundant family of Diptera in Neotropical Malaise catches (Brown 2005) and that many undescribed species are present in samples from Malaise, light and other traps (A. Borkent, pers. obs., Cannings 1994). Malaise material has been used to sample the less species-rich fungivorous subgroups (e.g., Jaschhof & Jaschhof 2009, 2013). Therefore, the number of species in even such otherwise well-collected areas as Britain, with 653 described species (Chandler 2017), will rise substantially once study of the group is undertaken using standard collecting methods. Relying solely on sequence data, Hebert *et al.* (2016) recently reported the presence of 8,467 species of cecidomyiids (as BINs: Barcode Index Numbers) in their survey of Canadian localities (fewer than 200 named

species are reported otherwise) and projected from this an estimate of 16,000 for Canada and 1.8 or 2 million species worldwide, surpassing the total named diversity of the Insecta. Our results also suggest that a tremendous number of cecidomyiid species are indeed likely present on Earth. This is discussed further below.

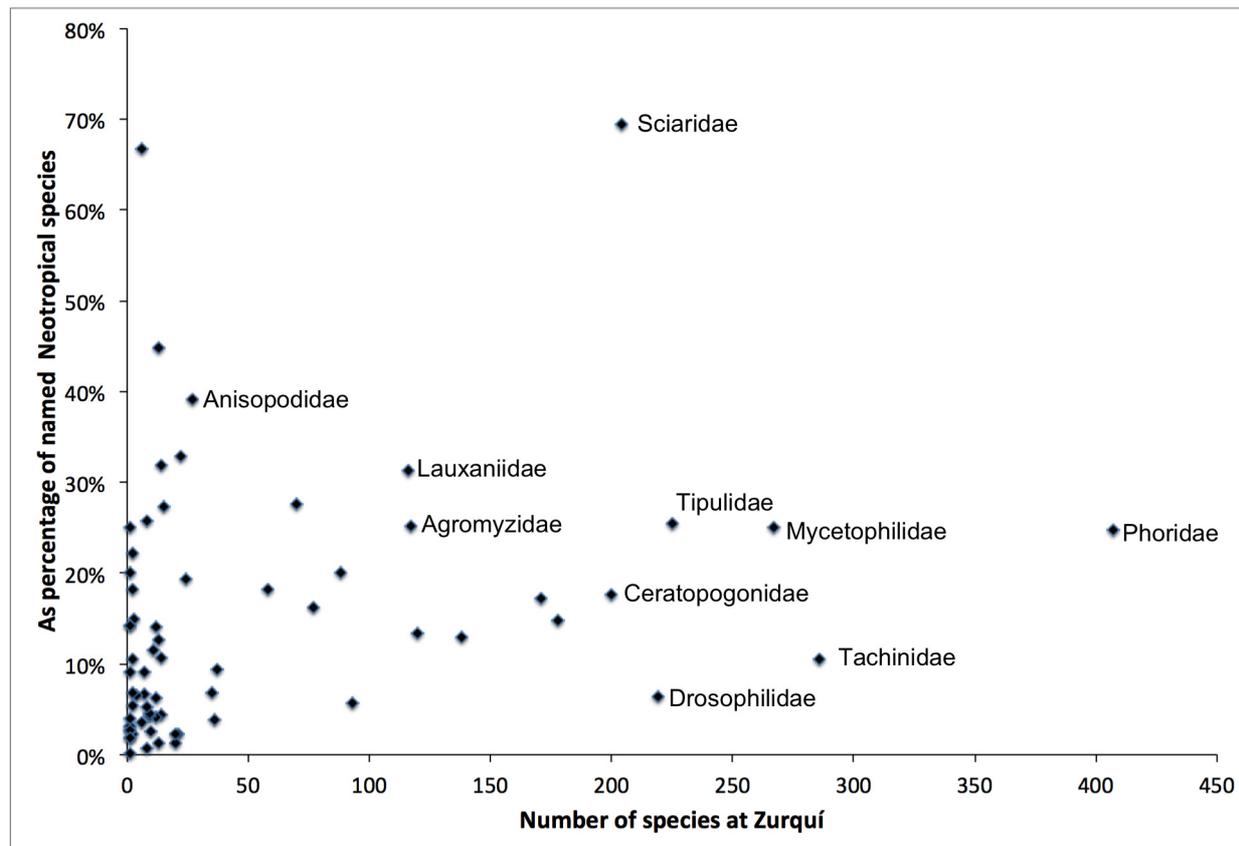


FIGURE 6. Relationship between number of all species occurring at Zurquí (named and unnamed) and all named species from the Neotropical Region (not including Cecidomyiidae, with 800 species). Numbers of species from Pape and Thompson (2013) with some modifications by coauthors.

Similarly, Hebert *et al.* (2016) identified the Sciaridae, with 2,277 putative Canadian species (as BINs), as the second most diverse family of Diptera in that country (30 named species reported). Our results also indicate the presence of much higher diversity of sciarids than otherwise reported in the Neotropical Region (Table 2, Fig. 6). Other taxa such as Drosophilidae, Phoridae, and Mycetophilidae, relatively depauperate in Canada, had proportionately larger numbers of species at Zurquí.

How many of the Zurquí species are undescribed. Our goal in estimating dipteran diversity at our study sites was to determine how many species were present, regardless of whether we could name them or not. As such, many taxa were recognized only as numbered morphospecies. For many groups, some of the morphospecies may be named but there was taxonomic uncertainty (or difficulty) in applying a name. For example, for Ceratopogonidae, with 4,278 specimens to examine, even potentially named species were mostly given morphospecies designation until these can be studied more carefully in the light of an often scattered and challenging literature. However, in at least a few relatively well known families, new species were recognized that provide insight into what is present at Zurquí. As noted above, two of 13 species of the relatively well known Culicidae were recognized as new. At least three of 10 Simuliidae were new species. The recently revised Corethrellidae (Borkent 2008) had seven species of which two were undescribed. As such, even these comparatively well-studied groups revealed unnamed diversity. All families with many species had large numbers of undescribed species although for most, these could not be exactly determined. However, Chironomidae were represented by 138 species, of which 98 (71%) were undescribed. Sciaridae were represented by 204 species, most of which were undescribed, including species of 13 undescribed genera. The Psychodidae with a total of 171

species included only 24 named, leaving 147 species (86% of the family) undescribed. Of the 314 species of Cecidomyiidae in the fungivorous subfamilies (Lestremiinae, Micromyinae, Winnertiinae, Porricondyliinae), seven were previously named and 307 (98%) were undescribed species. Of the latter, 24 (and three new genera) were named during the project (Jaschhof & Jaschhof 2014; Jaschhof 2016); 283 new species of these subfamilies remain unpublished. The remaining 486 species, in the subfamily Cecidomyiinae, are largely or possibly entirely unnamed. Within the Mycetophilidae, the genus *Mycetophila* had 136 species at Zurquí. Only four named species are known from Costa Rica, indicating that the vast majority of *Mycetophila* from Zurquí are unnamed. These observations on individual families confirm a startling level of undescribed diversity seen when comparing numbers of largely unnamed species from Zurquí with those named from increasingly broader areas (Table 2, Figs 4–7).

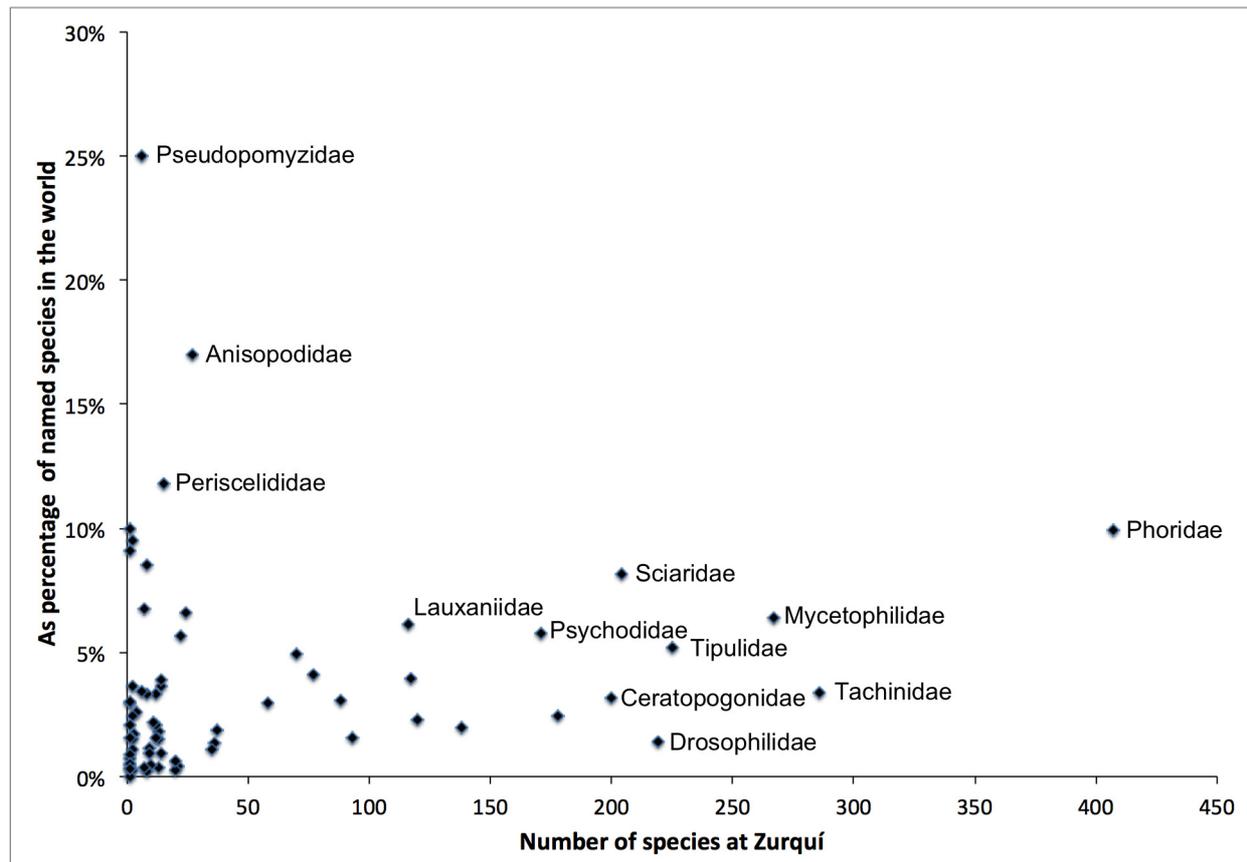


FIGURE 7. Relationship between number of all species occurring at Zurquí (named and unnamed) and all named species from the world (not including Cecidomyiidae, with 800 species). Numbers of species from Pape and Thompson (2013), except as noted in Table 2.

Discussion

When Linnaeus (1758) first published his list of the species of the world, he included 10 genera and 191 species of Diptera (Thompson & Pape 2016), a number he thought reflected nearly all there was to know (Linnaeus 1749). Clearly he underestimated the magnitude of this order and today we recognize 159,051 named species (Table 2). Various authors have considered Diptera to make up 12–15% of Hexapoda (Grimaldi & Engel 2005; Zhang 2011b), 10–15% of Animalia (Yeates & Wiegmann 2005; Zhang 2011a, b), or 10% of all life (Brown 2009). Inconsistencies in various estimates for many groups are partially due to the lack of reliable catalogs of described species in many groups. Regardless, our results strongly indicate that all previous estimates of dipteran diversity (except that by Hebert *et al.* 2016) are far off the mark. In reality, there are many more species yet to be described and, when they ultimately are, that will push this order to a much higher rank in terms of percentage of known animal life, compared to vertebrates and some of the better known groups of insects, such as butterflies.

How many species of Diptera are present on Earth. In spite of numerous previous attempts to estimate diversity worldwide, a high level of uncertainty remains regarding the numbers of species on our planet, whether one is considering an order of hyperdiverse insects, all arthropods, all eukaryotes, or even the totality of organismic life (Berenbaum 2009). Due to this uncertainty, various authors have applied an array of techniques to extrapolate from limited data to a worldwide basis (Borkent & Brown 2015). The literature is strewn with estimates of how many species of various groups are present on Earth and numerous authors have lamented the lack of accurate knowledge. Our project took the hard route to partially address the question by examining how many species of one of the hyperdiverse orders of insects were present in a mainland tropical habitat.

Some other studies sampling a given area have been more constrained by restricting their projections to a limited location. All of these, however, have selected certain taxa as representatives of a broader biota, using various models (mostly species accumulation curves) to propose total diversity. Species accumulation curves are powerful tools to interpret local diversity but will fail to accurately estimate broader species numbers if too few representative groups are used in the analysis. Nearly all have opted for breadth of taxa at the cost of setting aside the taxonomically "difficult" (often most species-rich) groups. Basset *et al.* (2012) estimated a total of 25,000 species of arthropods for a Panamanian reserve of 6,000 hectares based on 6,144 species sampled from 0.48 hectares. For the Diptera, they extrapolated from 193 species in only four families to suggest the presence of either 1,429 or 1,754 species of Diptera (depending on extrapolation method). These figures are in stark contrast to our count of 4,332 species at Zurquí and indicate that Basset *et al.* (2012) seriously underestimated species numbers as a result of their very restricted taxon sampling.

The use of surrogate taxa to estimate total diversity is employed in virtually all temperate studies as well (e.g., Scherber *et al.* 2014) and, in ignoring the high diversity of small and often delicate species, reflects a serious gap in our understanding of community structure and function everywhere. For example, Báldi (2003) argued that family richness reflected species richness in Diptera. Our results show that this conclusion is flawed and that interpreting diversity at the species level, including those groups which are generally neglected, is vital (Wolters *et al.* 2006).

A recent study of tropical Diptera in Australia (Smith & Mayfield 2015) compared numbers of species of Diptera in patches of wet lowland forest of varying sizes. Their identification of 172 morphospecies in 33 families might suggest low diversity in those habitats. However, their sampling was strongly constricted, collecting three times at each of 35 sites (20 x 20 m), using nine pan traps for 24 hours and aerial netting for 7.5 minutes. The sampling period was for only two months (during the wet season). The nematoceros Diptera included, for example, only 21 species in five families. As such, it is highly probable that the dipteran fauna in those tropical forest patches was significantly higher.

The diversity of Diptera at Zurquí was astoundingly high and with at least 4,332 species represents the most species-rich area of such limited extent yet discovered for this order anywhere on the planet. It is important to consider, however, that we selected this site because it had the following agreeable combination of scientific and logistical features. First, many of us had conducted research in Costa Rica, a particularly science-friendly tropical country to work in, for numbers of years. In conjunction with this, most of us were involved in the writing of the comprehensive *Manual of Central American Diptera* (Brown *et al.* 2009, 2011), which provided an excellent foundation for interpreting the species. Second, the Zurquí site is near the (sadly) now almost defunct Instituto Nacional de Biodiversidad where excellent facilities were available and where previously trained and highly skilled technicians were available to work on this project. Third, Zurquí had been previously recognized as a diverse site for Hymenoptera (e.g., Gaston & Gauld 1993; Gauld 1997; Hanson 1995) and Phoridae (Brown 1996, 2000). Fourth, as a mid-elevation site, it was thought more likely to have higher levels of endemism than a lowland site. Finally, much of the site at Zurquí was pristine cloud-forest that abuts Braulio Carrillo National Park (4,409,900 hectares). As such, we do not consider the huge diversity at Zurquí to be unusual, at least within most of the Neotropical Region.

There is still little understanding of why a particular lineage of Diptera diversifies in a certain area or in a particular habitat. The fauna of the Neotropical Region is extremely rich and this region, encompassing more than 75 degrees of latitude, is immensely varied in topography and climate. Other areas are more depauperate. For example, the subtropical and highly xeric United Arab Emirates, with 83,600 km², has been studied in some detail (van Harten 2008). As of April, 2017, a total of 711 species of Diptera have been recorded (at least 103 endemic) in 63 families (A. van Harten, pers. comm.). Eight of these families have not yet had their species identified. In addition, the Cecidomyiidae were incompletely surveyed (12 Lestremiinae, 3 Micromyiinae, 3 Cecidomyiinae

present; Harris & van Harten 2010; Jaschhof & Jaschhof 2011) which might add some but clearly not a huge number of species. Regardless, lower diversity would be expected, considering what we know of the general habitats of Diptera. Other tropical locations inventoried for Diptera are islands and have limited faunas as well (even though not all families were studied or incompletely so): Galápagos with 294 (Sinclair 2009), Hawaii with 1,518 (Evenhuis 2009), Canary Islands with 1,024 (Evenhuis 2009), Seychelles with 630 species (Gerlach 2009) and Fiji with 701 species (Evenhuis 2017). Studies of other mainland tropical sites are either dated and are clearly incomplete (e.g., Curran 1934) or had limited collecting and restricted taxon sampling and therefore are not comparable to our study (Basset *et al.* 2012; Stork 1991).

If various lineages of Diptera diversified at the same rate in terms of numbers of species, there would be some hope of utilizing the numbers of species of such relatively well known groups as Culicidae and Tabanidae in different regions as indicators of total dipteran diversity; this is unfortunately not the case. It is our opinion that projections of total numbers on Earth are not possible until we have a more complete inventory of at least the most species-rich groups from substantially more areas and different habitat types in at least the tropics. As it stands now, our knowledge is so limited that we will continue to flounder until such data are generated.

It is difficult to relate to non-specialists how vast our ignorance truly is. The Tachinidae are a case in point. Although there are 286 species at Zurquí, only 123 species are described from Costa Rica and 210 from all of Central America (Table 2; Wood & Zumbado 2011, with modifications since). Based on years of specialist sampling by D.M. Wood elsewhere in Costa Rica and Central America, there are an estimated 2,000 species in Central America, most of these undescribed (Wood & Zumbado 2011). The family has a worldwide total of 8,500 recognized species (Table 2). As we have shown here, other big Diptera families are in similar positions (Table 2, Figs 4–7), with huge diversity at Zurquí and comparatively poorly known elsewhere.

For decades, the Coleoptera has been the flagship group to compare diversity patterns and has functioned as a surrogate for tropical diversity in general (Stork 2018). Estimates that Coleoptera, with 386,500 named species (Zhang 2011b), represent 31% of all arthropods suggests that they are the most diverse order in the world. However, in temperate regions, named Diptera are often more diverse than Coleoptera with, for example, 7,141 Diptera and 4,069 Coleoptera in Great Britain (Duff *et al.* 2012; Chandler 2017). Moreover, the accumulation curves for Diptera (and Hymenoptera) suggest that the number of dipteran species will continue to rise for still some time, whereas the British Coleoptera fauna has been nearly stagnant since about 1900 (Gaston 1991). In the Nearctic Region, including the Nearctic portion of Mexico, there are 22,229 species of Diptera (Pape & Thompson 2013) and 25,160 species of Coleoptera in the more restricted area of Canada and the United States of America (Marske & Ivie 2003). The British and North American comparisons include many cecidomyiids but the number of species will markedly increase once this family is sampled with otherwise standard collecting methods and these will add significantly to the total number of dipteran species in temperate areas. An ongoing taxonomic inventory of fungivorous Cecidomyiidae in Sweden has identified 227 species new to science, of which 138 have been described so far (Jaschhof & Jaschhof 2009, 2013); it is presently unknown how many species of Cecidomyiinae are present there.

The big boost to numbers of Coleoptera comes from species in the tropics. Their body structure probably makes them the most amenable of the hyperdiverse orders of insects to collect and preserve, including those that are the smallest members of the order (Stork *et al.* 2015). This likely introduces a bias in relative appraisals of species richness in other groups (see Noyes 2012, for similar discoveries among small Hymenoptera). Our study included, for the first time for tropical Diptera, the families with the smallest and/or most fragile members, such as Cecidomyiidae and Chironomidae. Other diverse families, such as Ceratopogonidae, Mycetophilidae, Phoridae, Psychodidae, Sciaridae and Tipulidae are rarely included in broader studies due to either their small size, fragility, a lack of systematists, challenging identifications, lack of financial support, or most often, a combination of these factors. Ironically, because these groups are so diverse (and often inadequately described), they are avoided in virtually all ecological and other studies surveying taxa. Even though Malaise traps are routinely used in other insect surveys, the background "noise" of small and often broken specimens in samples is ignored. As such, it was precisely our determination to sift through those otherwise neglected taxa that allowed us to create the most complete picture of adult dipteran diversity at a mainland tropical site to date.

Our data strongly indicate that Diptera will likely surpass the Coleoptera in numbers of species worldwide, but our goal in this project was not to determine global diversity of the group (see below). Rather it was to catch the first focused glimpse of tropical dipteran diversity, as an initial gauge of our collective ignorance. Postulating total

diversity levels on the planet, as summarized in the introduction, will likely continue to vary widely until we have more evidence concerning such groups as Diptera in their breadth.

Virtually all biodiversity studies on holometabolous insects are limited to the adult stage, even though the primary functions of this stage are mostly restricted to reproduction and dispersal. It is the larvae that use a wide array of resources within given habitats. In our study, we do not know how many of the species recovered are actually using the site as habitat as opposed to being transients (e.g., those blown into the area). Adults may utilize resources, such as nectar and other food present at the site, without their immatures being present. Adults may also use an area as an important component of their dispersal. However, to understand the full ecology of such sites it must be part of future goals to determine where the immatures are living and what resources they use (Missa *et al.* 2009). Some of our trapping at Zurquí was directed at capturing emerging Diptera. Emergence traps over a wide array of substrates both terrestrial (e.g., leaf litter, bromeliads, branches both wet and dry, mushrooms) and aquatic (running, standing, stagnant) captured 769 species (Fig. 2), representing 18% of the total number of 4,332 species recorded at Zurquí.

Some studies have opted for examining levels of diversity using identifications only to the generic or family level (e.g., Báldi 2003; Kitching *et al.* 2004, 2005; Lambkin *et al.* 2011). In biodiversity studies it is often forgotten that taxon names above the species level are artificial and are primarily for the convenience of humans. Genera and families are categories grouping various lineages which are, or should be, monophyletic and which appear to have a morphological gap between them. Comparisons between non-sister groups or between groups of significantly different age are largely meaningless in a biological sense. Hennig (1950, 1966) pointed out long ago that only phylogenies provide a sound basis for logical comparisons between taxa. For example, of the four hyperdiverse orders of insects, if Diptera, Lepidoptera and Hymenoptera form a monophyletic group (Grimaldi & Engel 2005), they collectively include more than 429,676 species. Together they have been hypothesized as the sister group of Coleoptera with 386,500 species (Grimaldi & Engel 2005; Zhang 2011b). A historically based question, not pursued here, is "how did two sister groups diversify and how do they compare in their numbers of species, specializations and adaptations?"

How can there be 4,332 species of Diptera at Zurquí. The outstanding diversity of Diptera at Zurquí begs the question as to how so many species can live in such a small area of four hectares. It is well known for many groups that ecological specialization is widespread in the tropics. Condon *et al.* (2008), for example, found 52 Neotropical species of tephritid fruit flies in the genus *Blepharoneura* Loew on 24 cucurbit host plant species with high levels of host specificity as well as specificity to certain parts of the plants. Widespread plant species supported as many as 13 species of *Blepharoneura*, indicating that geography, age and area are also important components of diversity. Brown & Feener (1995) found 127 species of the ant-parasitizing phorid genus *Apocephalus* Coquillett at La Selva Biological Station in Costa Rica, most of which are specialized on single ant host species. Brown (1999) additionally found specialization of various phorid flies on specific parts of ants (i.e., one species on the head, one on the abdomen, one on the legs). Tachinidae also may be highly host specific (Smith *et al.* 2006, 2007). Pyrgotidae are considered to be strictly parasitoids of adult chafer beetles, with morphologically specialized modes of oviposition into host bodies not only of different size, but also of different sex (V. Korneyev, unpublished data). Other host-specific groups, such as the streblid flies that parasitize bats, are closely tied in distribution and diversity to their hosts. The Neotropical Region is especially diverse in bat species (particularly leaf-nosed bats of the family Phyllostomidae) and likewise streblid diversity reaches its zenith in the Neotropics (Dick & Patterson 2006). Habitat distinction on a small scale may also promote fly diversity. For example, substrate specialization appears key to sympatric diversity in the micro-Dolichopodidae (several genera with species about one mm in size, e.g., *Enlinia* Aldrich with seven species at Zurquí) since adults of some species occur only on soil or wet sand, others on leaves or tree trunks, and even different species on wet versus dry surfaces of the same rock (Robinson 1969). Every species-rich family of Diptera includes examples such as these.

Many of the most diverse groups at Zurquí are those that thrive in wet environments (e.g., Tipulidae, Ceratopogonidae, Chironomidae, Mycetophilidae, Sciaridae, Dolichopodidae, Psychodidae, Drosophilidae), habitats that were certainly abundant in this cloud forest. Other groups, like the diverse Tachinidae are parasitoids, generally on herbivorous insects, which are known to have high richness in tropical forests. The Zurquí site also was obviously amenable to the larvae of such plant-associated (for most species) families as Cecidomyiidae, Agromyzidae and Chloropidae and fungus-associated Mycetophilidae and Sciaridae. Sciaridae and Drosophilidae

larvae often live in decaying vegetative matter and fungi. The second largest group at Zurquí, the Phoridae, is so diverse and poorly-known that assigning its components to certain lifestyles and habitats is premature. Based on current knowledge, they include the third largest group of parasitoid Diptera (after Tachinidae and Bombyliidae), but mostly attacking social insects (especially ants), which are not as diverse at higher elevations as in the lowlands. Among the 407 recognized phorid species at Zurquí, there are ant, bee, termite, beetle, and millipede parasitoids and various commensals, non-predators, and non-parasitoids but the lifestyles of most are unknown.

Of all species collected during our study (other than 407 species of Phoridae—which were studied primarily only from Malaise trap #1), emergence traps sampled 769 species (360 of these unique to the project; Table 3), demonstrating that at least 18% of the 4,332 species of Diptera reported here were likely present as immatures at Zurquí. Of these the families Cecidomyiidae (n= 106), Chironomidae (n= 78), Dolichopodidae (n= 75), Sciaridae (n= 68), Psychodidae (n= 50), and Ceratopogonidae (n= 48) predominated. We do not know how many of the remaining species bred at the site, nor how many used the area only as a source of nectar or other adult resources, nor how many might have simply blown into the area by regional weather (and if that might be important for the dispersal of the species). Malaise trap #1 (Fig. 1A) located at the edge of the forest collected the greatest number of species and may have sampled more species blown into the area when strong winds, often common, were present at Zurquí. Regardless, discovery of the specializations and microhabitats that support this diversity will provide a wealth of future challenges.

Why not just DNA barcode all the species everywhere. Some readers will be surprised to find that we did not DNA barcode the species we studied morphologically, especially when it is clear that such information can provide important clarity regarding some species' identities (Janzen *et al.* 2009). This was due to financial and logistical limitations.

There are repeated claims that barcoding is a faster path to determining the identity and numbers of species (e.g., Hendrich *et al.* 2015; Kekkonen & Hebert 2014; Meier *et al.* 2006; Ratnasingham & Hebert 2013). Aside from issues regarding the interpretation of DNA barcodes for species recognition (Jaschhof 2010; Zahari *et al.* 2014), our goal was not only to determine how many species were present at Zurquí, but also to understand them on a broader level (Wheeler 1995). Morphological studies are far more informative in understanding diversity than a species known only as a number. Barcoding, of course, is a powerful and useful tool in aiding species identity (including for inventories) and association of sexes and poorly known immatures with adults but we see it as just one useful instrument in a much larger toolbox (e.g., Dénes *et al.* 2016; Germann *et al.* 2010; Heller & Rulik 2016; Jürgenstein *et al.* 2015; Kehlmaier & Assmann 2008; Petersen *et al.* 2007; Salmela *et al.* 2014; Ståhls & Haarto 2014; Stur & Borkent 2014; Stur & Ekrem 2015; Willassen 2005). Barcode clusters are hypotheses of species identity that need to be confirmed by taxonomists working with more complete sets of evidence.

Hebert *et al.* (2016) recently surveyed the Canadian insect fauna using just barcodes of specimens identified to family (some to genus), determining that Canada had 23,591 species of Diptera, nearly three times greater than those named (Langor 2015). The recognition of strikingly higher diversity of Cecidomyiidae, Chironomidae, Sciaridae, Ceratopogonidae, Mycetophilidae, and Phoridae than previously known clearly indicates that much of the Canadian fauna is poorly known for at least these families. However, the specimens of these families were cleared during the extraction process and are now stored in ethanol (other methods are not so damaging—Meier *et al.* 2016). Without further curation, these specimens may be lost to science as far as any further study of their morphology is concerned within a few decades (and perhaps sooner—at least some old alcohol specimens are impossible to slide mount for some families of Diptera). Further to this, examination of photos of barcoded specimens in the Barcode of Life Data Systems (BOLD; www.boldsystems.org) shows that some are in such poor condition there is little chance they could be studied morphologically. Many, and especially those of Cecidomyiidae, probably will be unsuitable for study by taxonomists (i.e., not subject to verification). There appear to be no plans by their project to curate this material (other than responding to taxonomists who wish to borrow specimens) and therefore the future identification of many of these species will require fresh material that might then be studied morphologically. Some of the Canadian specimens in other families were examined by specialists during the course of their study but there is no evidence in the publication of what contribution these specialists made (or their names).

Study of morphology by taxonomic experts provides further levels of information that help to address further biological questions. It is of limited value to know solely that there are piles of species in certain groups. When

genera and species are examined, named, and described, the information content provided increases markedly. Data on morphological differences between species, morphological adaptations, bionomics and phylogenetic relationships (including related species and their adaptations) then become available (Wheeler 1995). The Cecidomyiidae are a case in point. Recognition of 8,467 putative species of Canadian Cecidomyiidae (as BINs) is of limited value when these are only identified to the family level (Hebert *et al.* 2016). As pointed out above, there is a wealth of feeding modes and larval habitats within this family, which in conjunction with other adaptations are understood in major ways by systematists working primarily morphologically on the group (Gagné 1989, 1994; Jaschhof & Jaschhof 2009, 2013). This is true for all families of Diptera, with each having various adaptations and patterns of diversification understood, at least in part, by systematists. This knowledge of biodiversity not only informs our understanding today but is predictive for interpreting newly discovered patterns. It is, therefore, our consideration that had Hebert *et al.* (2016) incorporated the study of the material by systematists, the publication would have been markedly enhanced.

The question here, therefore, reflects our perspective on what our goals as biologists should be. Counting species is fine but these numbers in and of themselves are of limited value. What difference does it make to our science whether there are 3, 5 or 10 million unnamed species on Earth? The numbers by themselves are rather useless in their biological significance, other than watching extinction rates (Costello *et al.* 2013; Pimm *et al.* 1995). For instance, it is an interesting fact that there are more than 1,400 species of birds in Bolivia but that number does not provide much information in itself. However, a wealth of interesting questions arise when one knows their morphology, phylogenetic relationships (including incorporating fossils) and zoogeography, interpreting their functional morphology and adaptations, and understanding species interactions, life histories, and use of resources (by adults and juveniles). All of these are part of the patterns of routine interest to systematists. It is these sorts of questions that will help us better understand what factors drive tropical diversity.

A repeated point in the literature is that morphotaxonomy is expensive and DNA barcoding relatively cheap (e.g., Carbayo & Marque 2011; Hebert *et al.* 2016; Smith *et al.* 2008; Wong *et al.* 2014; Yu *et al.* 2012). So too, there is the argument that molecular techniques are fast, while it will take hundreds of years to describe all the species present on our planet. However, we often get what we pay for. As a group, our experience comprises 1,771 person years of taxonomic study of Diptera and, at the risk of boasting, we collectively know a great deal about the organisms we study that includes first hand contact with the organisms, in nature and in the laboratory, knowledge that is vital to a broader interpretation of biodiversity. Knowing the morphology (including barcoding), behaviour and general habitats of the taxa under study, therefore, clarifies the breadth and nature of inventories (Cardoso *et al.* 2011).

Where do we go from here? Suggestions for future research. Our one year study was limited, being restricted to two continuously running Malaise traps with a variety of other collecting methods used for three days per month at Zurquí and a continuous Malaise trap at each of Tapantí and Las Alturas. Further collecting is needed, especially for determining the asymptote on the species accumulation curve (Brown *et al.*, unpublished data). Important components in the interpretation of species inventories are the documentation of the distributions of the included species and the need for beta-diversity comparisons.

Here, all species were identified to named species or morphospecies (Appendix 1), nearly all of which require further taxonomic study and interpretation (but see papers generated by our project: Adler *et al.* 2017; Bickel 2015; Brown 2014; Brown & Porras 2016; Costa *et al.* 2016; Epler 2017; Grimaldi 2016; Hartop & Brown 2014a, b, c; Jaschhof 2014, 2016; Jaschhof & Jaschhof 2014, Kvitte *et al.* 2016, 2018; Marshall 2015; Michelsen 2017; Santarém *et al.* in press). ZADBI specimens will undoubtedly form the basis for many more taxonomic papers over the coming decades. This clearly reflects the current general lack of comprehensive monographs that can be used as identification guides. Our study included only adults (other than immatures of Simuliidae), and in some instances, only males of certain genera. For the vast majority of species the larval stage is of the greatest duration and is the stage in which most feeding (and thus ecological importance) is concentrated. As such, documentation of larval habitats will substantially enhance our understanding of community structure (and inventories).

Erwin (1991, 2004) and Erwin *et al.* (2005) proposed a research model that would systematically collect and interpret tropical diversity. Primarily it is a call for the investigation of species through beta-diversity studies over a grid of 1° by 1° over the face of the earth. We agree. It is our contention that future estimates of species diversity should be based on actual study of the species on our planet and that, as Erwin (2004) noted, we should move from

a "model rich, data poor" situation to undertaking the science that needs to be done, to truly identify, interpret and protect the fauna (Cardoso *et al.* 2011; Erwin & Geraci 2009). What is required is an army of systematists and technicians (including those skilled in DNA barcoding and other molecular techniques), carefully selected and constrained sampling areas throughout the tropical regions of the world, and the societal vision to support such an endeavour before what will certainly be the extinction of literally untold numbers of species. Although papers providing conjectures regarding total species numbers on the planet will likely continue to be published and can appear to be "big science", the reality is that we will not know until we look.

Our goal as biologists is to understand life in all its breadth. The role of systematists is fundamental to this endeavour in as much as we describe species (using morphological, behavioural, bionomic and molecular data) and provide evolutionary trees that provide the basis for interpreting all biological patterns. Species need to be named (placed into a historical context with their phylogenetic relations) and ecological roles and morphological adaptations need interpretation and description. The Diptera are one of the most exciting and promising groups to study because they are remarkably diverse in terms of species, morphology and ecology. Flies are abundant and broadly distributed and further intense study would almost certainly lead to future discoveries that would match the wealth of information that came from discovering polytene chromosomes in *Drosophila*, the role of Culicidae and other biting flies in the transmission of diseases, and a host of other outstanding and valuable biological phenomena.

The need for basic biosystematic research extends throughout the planet. Although we have discovered an enormous level of dipteran diversity in a cloud forest in Costa Rica, Fontaine *et al.* (2012) showed that even Europe is incompletely understood, with the Diptera fauna there not yet reaching an asymptote (also from data provided by B. Fontaine, pers. comm.). The Neotropical and Oriental Regions are likely the richest biogeographic areas in the world for Diptera and also the most poorly known (Amorim 2009; Brown 2005; Grootaert 2009). At a time when most science funding goes to applied or highly directed research, there is clearly a tremendous need for basic research if we are to explore the richness of dipteran diversity. As all other species-related research relies on the recognition of species, it is of paramount importance that this basis (taxonomic research) is strong and maintained. Our present knowledge is so poor, however, that only the most general of zoogeographic patterns are known in the New World tropics (Amorim 2009). For family after family, we see the same pattern of virtually or entirely unexplored faunas throughout much of Central and South America. We are truly the early explorers on much of the planet.

Human beings living in the 21st century are facing a frightening paradox. There is a broad scientific consensus that we are currently experiencing a sixth extinction of epic proportions, based in large measure on data from vertebrates (Dirzo *et al.* 2014; Kolbert 2014). On the other hand, it is widely understood that we haven't named, nor even collected, the majority of species and do not know how many, by orders of magnitude, are living on our planet (e.g., Larsen *et al.* 2017). Costello *et al.* (2013), however, suggested that extinction rates are not of the alarming magnitude that some believe them to be because they estimate the presence of 5 ± 3 million eukaryotes and with substantial taxonomic effort progress being made to describe them. Before accurate estimates of diversity and extinction rates can be determined, it is imperative to understand the true diversity of species. Detailed inventories and understanding distributions of those species are of vital importance to more precise estimates (Wiens 2016). Our study shows strikingly high diversity of Diptera and likely high levels of endemism. If endemism is indeed elevated, especially at higher altitudes, species are very likely disappearing at an equally high rate, based on the reasonable assumption that clear-cut tropical mountains once held large numbers of endemic species. For example, we will now never know how many species once occupied the forests of Haiti, and how many of those were unique, before that country was nearly entirely denuded of its native vegetation.

Approximately 1.9 million species of metazoan life have been named thus far (Chapman 2009) and estimates of the number of named and unnamed insect species generally range from 1.8 to 10 million. Among conservationists, the focus has been on attempts to save various mammals, birds, and amphibians while the status of invertebrates, with the exception of some butterflies, remains very poorly understood (Berenbaum 2009; Dirzo *et al.* 2014). Such ignorance is unsettling because insects, which presently make up about 40% of all named eukaryotic life, are the "movers and shakers" of terrestrial ecosystems. We know that we are causing significant harm to our planet but we have little idea of what impact that has on those insect species upon which we depend (e.g., pollinators, decomposers, food for many vertebrates, etc., etc.). How can we manage and protect other species that co-inhabit Earth when we do not even know what they are or where they live? Such ignorance not only

impacts conservation decisions but also points to the wealth of untapped information yet available for numerous areas of biological research, including fundamental questions of evolution, community structure, morphology, and development, among many others.

Since Galileo's first forays into examining the plethora of stars that are in our universe, many billions of dollars have been spent on furthering our understanding of the nature of those celestial bodies. So too, many billions have been spent on studying the details of matter itself. The failure to prioritize, or at least equalize, the study of biodiversity here on Earth is a gross failure to appreciate that the door is rapidly closing on our chance to comprehensively understand life on our planet. As valuable and intriguing as both celestial bodies and the nature of matter are, learning more is not critical to maintaining life on our planet. We are running out of time to apply substantial and sustained support to our understanding of the life forms that surround us and upon which we as a species depend.

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