Zootaxa 5555 (3): 331–384
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5555.3.3
http://zoobank.org/urn:lsid:zoobank.org:pub:9F799B78-9985-4AFB-AADD-32E0752997A3

Looking Into the Abyss—How Many Species of Biting Midges (Diptera: Ceratopogonidae) Are There? Their Remarkable Diversity in Costa Rica and Elsewhere

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Abstract

The biting midges (Ceratopogonidae) are one of the most species-rich families of insects on the planet with over 6,200 named species. However, their true diversity is unknown and this paper is the first to address the question. Our systematic study of the family in Costa Rica indicates that 192 species were present in a four hectare area of cloudforest at Zurquí de Moravia, at 1,600 m after a year of intensive sampling. Combined with a collection from a single Malaise trap at Tapantí for one year, about 40 kms away and also at 1,600 m, the total was 245 species with significant differences between the two areas and with the strong majority unnamed. This compares to 430 named species for all of Costa Rica and 1,314 for the entire Neotropical Region. Barcoding of 221,407 specimens from Costa Rica similarly indicates large numbers of unnamed species with 4,023 BINs present. On this basis, we project at least 5,000 species in Costa Rica and using ratios of named species here and elsewhere, we suggest that nearly 73,000 are present worldwide. Details from Malaise traps in the Área de Conservación Guanacaste also indicate various levels of endemism. Samples from Bolivia support an interpretation of high diversity.

The diversification of the family was examined by comparing phyletic lineages, rather than merely comparing numbers of species in various genera, providing insight as to why some lineages are more diverse than others. Zoogeographic patterns of named species suggest stronger southern connections for Costa Rican Ceratopogonidae in both cloudforest habitats as well as the country as a whole, although many are also more broadly distributed north and south of the country. Comparisons between various collecting methods at Zurquí de Moravia indicate the efficacy of Malaise traps but also the importance of light traps and other methods in sampling adults of Ceratopogonidae. Phenological data from the Malaise traps in the Área de Conservación Guanacaste suggest some patterns of emergence of adults in Costa Rica, the first for any tropical country anywhere.

Accepted by K. Moulton: 8 Oct. 2024; published: 19 Dec. 2024

Resumen

Las mosquitas picadoras (Ceratopogonidae) son una de las familias de insectos más ricas en especies del planeta, con más de 6,200 especies nominadas. Sin embargo, se desconoce su verdadera diversidad, y este artículo es el primero en abordar esta cuestión. Nuestro estudio sistemático de la familia en Costa Rica indica que 192 especies estaban presentes en un área de cuatro hectáreas de selva nublada en Zurquí de Moravia, a 1,600 m, después de un año de muestreo intensivo. Combinado con una colección de una única trampa Malaise en Tapantí durante un año, a unos 40 kms de distancia y también a 1,600 m el total fue de 245 especies, con diferencias significativas entre las dos áreas y la gran mayoría aún no nominada. Esto se compara con 430 especies nominadas para todo Costa Rica y 1,314 para toda la Región Neotropical. Los códigos de barras de 221,407 especímenes de Costa Rica indican de manera similar, un gran número de especies no nominadas con 4,023 BIN presentes. Sobre esta base, proyectamos al menos 5,000 especies en Costa Rica y utilizando proporciones de especies nombradas aquí y en otros lugares, sugerimos que casi 73,000 están presentes en todo el mundo. Los detalles de las trampas Malaise en el Área de Conservación Guanacaste también indican varios niveles de endemismo. Las muestras de Bolivia apoyan una interpretación de alta diversidad.

La diversificación de la familia se examinó comparando linajes filéticos, en lugar de simplemente comparar números de especies en varios géneros, lo que proporcionó información sobre por qué algunos linajes son más diversos que otros. Los patrones zoogeográficos de las especies nominadas sugieren conexiones más fuertes hacia el sur para los Ceratopogonidae costarricenses, tanto en los hábitats de las selvas nubladas como en el país en su conjunto, aunque muchos también están distribuidos más ampliamente al norte y al sur del país. Las comparaciones entre varios métodos de recolección en Zurquí de Moravia indican la eficacia de las trampas Malaise, pero también la importancia de las trampas de luz y otros métodos en el muestreo de adultos de Ceratopogonidae. Los datos fenológicos de las trampas Malaise en el Área de Conservación Guanacaste sugieren algunos patrones de aparición de adultos en Costa Rica, los primeros para un país tropical.

Key words: biodiversity, new species, zoogeography, aquatic, no-see-ums, Culicomorpha, ceratopogonid, biodiversity, phenology, tropical

Introduction

As we are currently experiencing the sixth extinction (Kolbert 2014), particularly evident among insects, the actual extent of the biodiversity remaining is a pressing and pertinent question. There are approximately 1.9 million named species of Metazoa but estimates of the true number of species on our planet generally range from 3–10 million, with some estimates going much higher (Borkent & Brown 2015). Such ignorance is entirely a reflection of the lack of study and concomitant lack of public support for the systematics of insects. The loss of this undescribed diversity, called by some "dark taxa" (Hartop *et al.* 2022, Meier *et al.* 2022), will certainly be a major lament of future generations who will be missing so much of the amazing results of explosive evolution that our current world is yet hanging on to.

Within this context of ignorance, one the most urgent gaps is our lack of understanding of the number of species of small organisms and their distribution in the tropics. Although it has been long understood that tropical regions have far more species than do temperate areas, the true scope of that diversity has been vigorously argued for some time, often with a model rich but data poor basis. This paper is an initial effort to address this problem for some tropical Ceratopogonidae, commonly known as biting midges or no-see-ums, for the first time for this family of flies.

There are four orders of insects that hold the bulk of metazoan terrestrial life with these being the Coleoptera, Hymenoptera, Lepidoptera and Diptera. Diptera alone comprise about 10.2% of named species of animal life. Within this huge order, Ceratopogonidae are consistently reported as one of the most common and ubiquitous families of Diptera caught by traps in the world (e.g. Brown 2005, Stork 1991). Srivathsan *et al.* 2023 sampled insects with 39 Malaise traps placed in five biogeographic regions, eight countries and numerous habitats and barcoded >225,000 specimens belonging to >25,000 species in 458 families. Ceratopogonidae were found to be the second most species rich family in the entire Insecta, after the remarkably diverse fly family Cecidomyiidae. Even though they are so incredibly diverse, the family suffers from lack of taxonomic study in nearly every region of the globe, in spite of a small group of dedicated systematists. How many hundreds of thousands of species of Diptera and in the case being addressed here, of Ceratopogonidae remaining to be described is largely unknown.

In an attempt to address of the question of diversity for Diptera, the ZADBI project (Zurquí All Diptera

Biodiversity Inventory) was undertaken in Costa Rica in 2012 (Brown *et al.* 2018, Borkent *et al.* 2018). The project included a year-long study of a four hectare site of tropical cloud forest at 1,600 m, located at Zurquí de Moravia, San José Province, Costa Rica (hereafter referred to as Zurquí) (Fig. 1) by a group of 59 systematists, each of whom was an expert in one or more of the 73 families of flies present. All Diptera sampled primarily with two Malaise traps but supplemented with a host of other traps, were identified to morphospecies, resulting in an astounding 4,332 species. Some limited comparisons were made with two other cloud forest sites, approximately 40 kms (Tapantí) and 180 kms (Las Alturas) distance from the primary site and each with a single Malaise trap. These traps were located 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). An overview of the project and its protocol was provided by Borkent & Brown (2015). Although a list of species was included as an appendix with Borkent *et al.* (2018), the vast majority were only identified to genus and given a number representing a morphospecies. It would have been beyond the scope of the project and far too costly in time for authors to compare material with type specimens and discuss the issues with available keys and other taxonomic issues. Since then, a few families have been interpreted in more detail and these are discussed below.

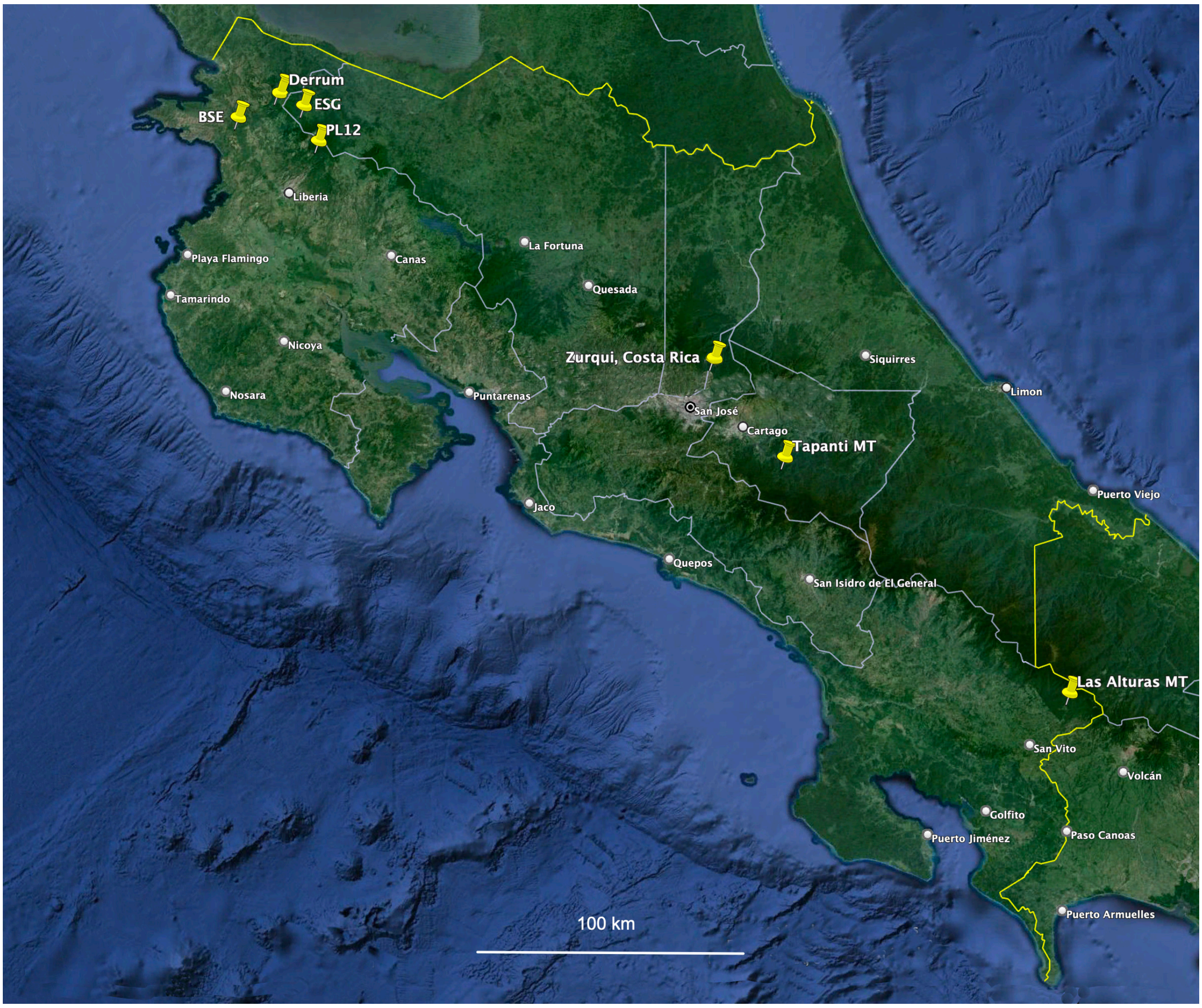

FIGURE 1. Map of Costa Rica showing location of the ZADBI sites at Zurquí, Tapantí and Las Alturas and those from the Área de Conservación Guanacaste BOLD analysis. Abbreviations as follows: BSE: Bosque San Emilio, Guanacaste Province; ESG: San Gerardo, Alajuela Province; PL12: Geothermal development platform PL12, Guanacaste Province; Derrum: Derrumbe, Guanacaste Province; MT: Malaise trap. Based on map produced with Google Earth.

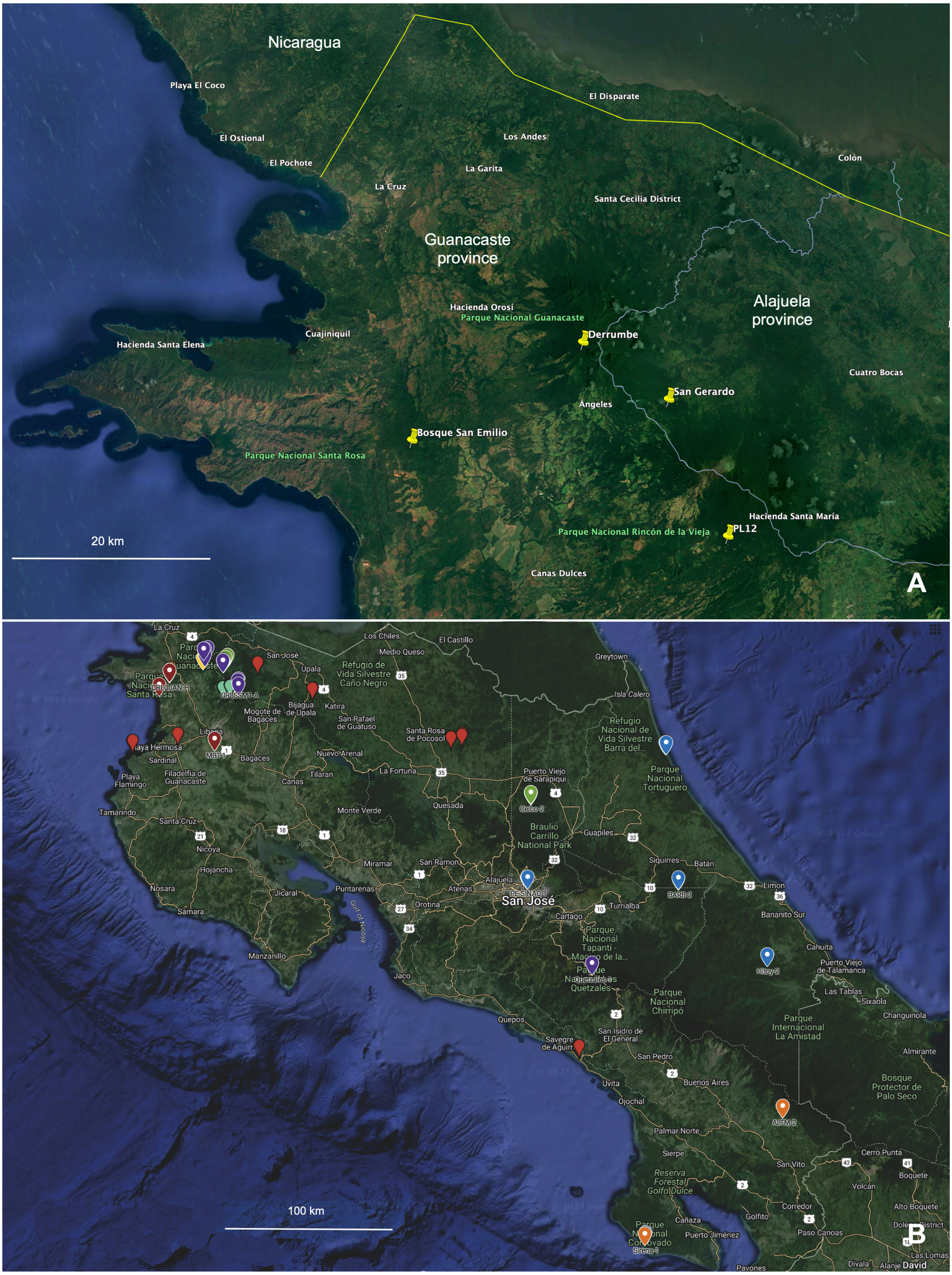

FIGURE 2. Maps showing location of Malaise traps for the BOLD analysis. A. Locations of the sampling in the Área de Conservación Guanacaste, Costa Rica indicated with yellow pin symbols. B. Locations of Malaise trap sampling throughout Costa Rica (variously coloured locations refer to data irrelevant here). Based on maps produced with Google Earth.

One of the predominant families present in that dipteran diversity was the Ceratopogonidae. As already noted, this is a huge family of flies, with over 6,200 named species placed in 109 genera (Borkent 2024, Borkent & Dominiak 2020, Borkent *et al.* 2022). Based on named species, worldwide it is presently the seventh most species rich family of Diptera and the eighth most diverse family at Zurquí, with exactly 200 species recorded by Borkent *et al.* (2018) and Brown *et al.* (2018). They occur in virtually all terrestrial areas on Earth, from coastal areas to high mountain peaks (to an elevation of least 4,400 m on Mt. Everest, Nepal), and from the tropics to the high arctic (within 150 kms of permanent polar ice) and subantarctic islands. Adults are one of the most abundant dipteran families represented in most insect traps and are particularly common in Malaise, light and other trap collections (e.g., Bickel & Tasker 2004: 892; Brown 2005; Kitching *et al.* 2004; Meiswinkel *et al.* 2004a: 95). The immatures are primarily present in at least moist to semi- and fully aquatic environments including such habitats as wet moss, rotting vegetation, phytotelmata, rock pools, seeps, margins of all lotic and lentic waters, the benthos of lakes and much more. The larvae of most feed on microorganisms or are predators of other invertebrates. Adults of many are pollinators as they feed on nectar and adult females feed on the blood of a remarkable range of hosts, from all major groups of vertebrates (including fish) to numerous forms of arthropods (primarily insects). A major group of genera feeds on the liquified contents of their insect prey (some from the males with which they are copulating) by injecting a proteolytic enzyme. This remarkable diversification makes them important in many ecosystems and excellent candidates for the study of diversification and evolutionary patterns.

Borkent (2017) suggested that there are at least 9,000 species of Ceratopogonidae that remain unnamed, based on morphological differences alone. Yet, there has been no published evidence of the extent of that unnamed diversity other than the first author's decades long experience perusing museum collections and examining many hundreds of thousands of specimens from around the globe. This paper provides a more detailed analysis of the Ceratopogonidae from the ZADBI project, interpreting those species from Zurquí and Tapantí, the two primary sites and comparing this to other sites in Costa Rica and elsewhere. In addition, there have been major efforts to DNA barcode Ceratopogonidae from Costa Rica and here we particularly pay attention to those from Guanacaste and other provinces based on Malaise trapping.

From this limited database, we speculate on the true diversity of this remarkable family of flies, in Costa Rican cloud forests, in the Área de Conservación Guanacaste (hereafter ACG) (Figs. 1, 2) and in the country as a whole, with comparisons made to other tropical sites in the Neotropical Region and to temperate areas elsewhere. Comparisons between collecting methods, the temporal distribution of the species at Zurquí and the relative numbers per species at Zurquí are also discussed.

Materials & Methods

The protocol and methods employed for the ZADBI study (Zurquí All Diptera Biodiversity Inventory) were described in detail by Borkent & Brown (2015) and Borkent *et al.* (2018). What follows is a summary of particularly pertinent aspects germane to our analysis and discussion here.

The primary study site of the ZADBI project was a four hectare patch of cloud forest at Zurquí de Moravia, San José Province, Costa Rica. The majority of the sampling, from Sept. 12, 2012–Oct. 18, 2013 included two permanent Malaise traps, one at the forest edge and one in a ravine near a small permanent stream. This was supplemented with a variety of trapping methods for three full days every month 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 and a canopy Malaise trap. Sweeping with an aerial net added some material and was performed periodically by the first author (weather and travel permitting). A permanent Malaise trap was placed at Tapantí National Park, Cartago Province (9.720°N, 83.774°W, 1,600 m), 40 kms from Zurquí, and provided comparative data. The Malaise trap at Las Altura, 180 kms southeast from Zurquí and reported for the more inclusive ZADBI study, retrieved too few specimens of Ceratopogonidae to be significant here (but its diversity is noted below).

Of the huge number of specimens collected in ethanol, select specimens deemed to be even slightly different within a given sample while yet in ethanol were slide mounted using the method described by Borkent & Spinelli (2007). As such, numbers of specimens per species were not recorded, although it appears likely that the numbers of slides per species is at least a broad reflection of relative abundance. A total of 4,281 specimens on microscope slides were studied, here tabulated for each genus (Table 1). The males of some genera provide more reliable morphological differences than females and a decision was made at the beginning of the project that the females of some genera (e.g. *Dasyhelea* Kieffer, *Atrichopogon* Kieffer, *Forcipomyia* Meigen) would not be examined further. Some genera were restricted to one sex because they were the only stage captured (i.e. *Alluaudomyia* Kieffer, *Parabezzia* Malloch, *Amerohelea* Grogan & Wirth, *Stenoxenus* Coquillett).

TABLE 1. Number of specimens of each genus from the three ZADBI sites and which sex was studied to determine morphospecies. Subfamilies and tribes are in phyletic sequence. Of the sex studied, m refers to males, f to females.

Subfamily	Tribe	Genus	sex studied	Zurquí	Tapantí	Las Alturas	Total
Forcipomyiinae	Dasyheleini	*Dasyhelea*	m	132	86	6	224
	Forcipomyiini	*Atrichopogon*	m	254	207	15	476
		Forcipomyia	m	793	753	22	1568
Ceratopogoninae	Culicoidini	*Culicoides*	m, f	1124	41	9	1174
	Ceratopogonini	*Alluaudomyia*	m	2	0		2
		Baeohelea	m	57	15		72
		Brachypogon	m, f	20	89		109
		Downeshelea	m, f	11	13		24
		Monohelea	m, f	48	27		75
		Schizonyxhelea	m, f	3	5		8
		Stilobezzia	m, f	112	22		134
	Parabezziini	*Parabezzia*	f	1	1		2
		Spinellihelea	m, f	1	18		19
	Echinoheleini	*Echinohelea*	m, f	15	7		22
	Heteromyiini	*Clinohelea*	m, f	2	8		10
	Palpomyiini	*Amerohelea*	f		5		5
		Bezzia	m, f	149	50	2	201
		Palpomyia	m, f	106	46	3	155
	Stenoxenini	*Stenoxenus*	m		1		1
		Total		2830	1394	57	4281

As a limited part of the study of the ZADBI Ceratopogonidae, an attempt was made to DNA barcode 95 specimens of *Culicoides* Latreille at the Centre for Biodiversity Genomics, University of Guelph, Canada with partial cytochrome c oxidase subunit 1 (COI) sequences of 86 specimens being produced. Eighty-four of these were complete and informative (Table 2, those noted with an asterisk).

In this study, our determination of named and unnamed species was limited and requires comment. We did not check the ZADBI material against types, which would have been ideal. In referring to published literature, with its inherent limitations for most genera, and to specimens from elsewhere in our own collections, it was apparent that some species from Zurquí were very near to previously described species with what in our estimation would be only minor morphological differences. In such instances, we have underlined these species names in Table 2 to indicate the presence of such variation. Further to this, keys to some Neotropical genera were severely dated and are in need of major revision (e.g. *Dasyhelea*, *Atrichopogon*, *Stilobezzia* Kieffer, *Palpomyia* Meigen), making confident identifications nearly impossible.

To calculate numbers of named species from Costa Rica, we included species known from this country, those known only from Panama or of species known only from both these countries (Borkent & Spinelli 2007; with the addition of more recent publications). These two countries generally have many shared species otherwise and similar continuity of habitat. A number of species are known from both north and south of Costa Rica but are not recorded from Costa Rica or Panama and these too were considered part of the Costa Rican + Panamanian fauna. We excluded species from Colombia because we lacked time to determine the exact locality from which species are known in that country. In our opinion, those from the Pacific coast seem more likely to also occur in Costa Rica and those known only from high altitudes and from the Amazonian side of Colombia are less likely to extend their distributions into Costa Rica.

TABLE 2. Identification of species from Zurquí and Tapantí, Costa Rica. Underlined species names indicates the possibility of misidentification. Species noted with an asterisk were barcoded. Subfamilies and tribes are in phyletic sequence.

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
Forcipomyiinae	Dasyheleini	*Dasyhelea*	*D.* (*Dasyhelea*)	*D. hamula*		x		Dasyhelea ZUR-17
			D. (*Pseudoculicoides*)		n. sp.	x	x	Dasyhelea ZUR-1
			D. (*Pseudoculicoides*)		n. sp.	x		Dasyhelea ZUR-12
			D. (*Pseudoculicoides*)		n. sp.	x		Dasyhelea ZUR-13
			D. (*Pseudoculicoides*)		n. sp.	x		Dasyhelea ZUR-14
			D. (*Pseudoculicoides*)		n. sp.	x	x	Dasyhelea ZUR-15
			D. (*Pseudoculicoides*)		n. sp.	x		Dasyhelea ZUR-16
			D. (*Pseudoculicoides*)		n. sp.	x		Dasyhelea ZUR-20
			D. (*Pseudoculicoides*)		n. sp.	x		Dasyhelea ZUR-21
			D. (*Pseudoculicoides*)		n. sp.	x		Dasyhelea ZUR-3
			D. (*Pseudoculicoides*)		n. sp.		x	Dasyhelea ZUR-34
			D. (*Pseudoculicoides*)		n. sp.		x	Dasyhelea ZUR-35
			D. (*Pseudoculicoides*)		n. sp.	x		Dasyhelea ZUR-4
			D. (*Pseudoculicoides*)		n. sp.	x	x	Dasyhelea ZUR-7
			D. (*Pseudoculicoides*)		n. sp.	x	x	Dasyhelea ZUR-8
			D. (*Pseudoculicoides*)?		n. sp.		x	Dasyhelea ZUR-36
			D. (Pseudoculicoides) (fasciigera species group)		n. sp.	x		Dasyhelea ZUR-11
			D. (*Pseudoculicoides*)?		n. sp.	x		Dasyhelea ZUR-2
			D. (*Sebessia*)		n. sp.	x	x	Dasyhelea ZUR-23
			D. (*Sebessia*)?		n. sp.		x	Dasyhelea ZUR-26
			leptobranchia species group	*D. patrycjae*		x	x	Dasyhelea ZUR-18
			leptobranchia species group		n. sp.	x		Dasyhelea ZUR-10
			leptobranchia species group		n. sp.		x	Dasyhelea ZUR-27

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TABLE 2. (Continued)

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
			leptobranchia species group		n. sp.		x	Dasyhelea ZUR-30
			leptobranchia species group		n. sp.		x	Dasyhelea ZUR-32
			leptobranchia species group		n. sp.		x	Dasyhelea ZUR-33
			leptobranchia species group		n. sp.	x		Dasyhelea ZUR-9
			Unplaced to species group		n. sp.	x		Dasyhelea ZUR-19
			Unplaced to species group		n. sp.	x		Dasyhelea ZUR-22
			Unplaced to species group		n. sp.		x	Dasyhelea ZUR-31
			Unplaced to species group		n. sp.	x	x	Dasyhelea ZUR-6
	Forcipomyiini	*Atrichopogon*		*A. colossus*		x		Atrichopogon ZUR-1A
				A. granditibialis			x	Atrichopogon ZUR-1B
					n. sp.	x	x	Atrichopogon ZUR-1C
					n. sp.	x	x	Atrichopogon ZUR-2
					n. sp.	x	x	Atrichopogon ZUR-3
					n. sp.	x		Atrichopogon ZUR-4
					n. sp.	x	x	Atrichopogon ZUR-5
					n. sp.	x	x	Atrichopogon ZUR-6
					n. sp.	x	x	Atrichopogon ZUR-7
					n. sp.	x		Atrichopogon ZUR-8
					n. sp.	x		Atrichopogon ZUR-9
					n. sp.	x		Atrichopogon ZUR-10
					n. sp.	x		Atrichopogon ZUR-11
					n. sp.	x		Atrichopogon ZUR-12
					n. sp.	x		Atrichopogon ZUR-13
					n. sp.	x		Atrichopogon ZUR-14
					n. sp.	x		Atrichopogon ZUR-15

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TABLE 2. (Continued)

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
					n. sp.	x		Atrichopogon ZUR-16
					n. sp.	x		Atrichopogon ZUR-17
					n. sp.	x		Atrichopogon ZUR-18
					n. sp.	x		Atrichopogon ZUR-19
					n. sp.	x		Atrichopogon ZUR-20
					n. sp.	x	x	Atrichopogon ZUR-21
					n. sp.	x		Atrichopogon ZUR-22
					n. sp.	x		Atrichopogon ZUR-23
					n. sp.	x		Atrichopogon ZUR-24
					n. sp.	x		Atrichopogon ZUR-25
					n. sp.	x		Atrichopogon ZUR-26
					n. sp.	x		Atrichopogon ZUR-27
					n. sp.	x		Atrichopogon ZUR-28
					n. sp.	x	x	Atrichopogon ZUR-29
					n. sp.	x		Atrichopogon ZUR-30
					n. sp.	x		Atrichopogon ZUR-31
					n. sp.	x	x	Atrichopogon ZUR-32
					n. sp.	x		Atrichopogon ZUR-33
					n. sp.	x		Atrichopogon ZUR-34
					n. sp.	x		Atrichopogon ZUR-35
					n. sp.	x		Atrichopogon ZUR-36
					n. sp.	x		Atrichopogon ZUR-37
					n. sp.	x		Atrichopogon ZUR-38
					n. sp.	x	x	Atrichopogon ZUR-39
					n. sp.	x		Atrichopogon ZUR-40

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TABLE 2. (Continued)

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
					n. sp.	x	x	Atrichopogon ZUR-41
					n. sp.		x	Atrichopogon ZUR-42
					n. sp.		x	Atrichopogon ZUR-43
					n. sp.		x	Atrichopogon ZUR-44
					n. sp.		x	Atrichopogon ZUR-45
					n. sp.		x	Atrichopogon ZUR-46
					n. sp.		x	Atrichopogon ZUR-47
					n. sp.	x	x	Atrichopogon ZUR-48
					n. sp.		x	Atrichopogon ZUR-49
					n. sp.		x	Atrichopogon ZUR-50
					n. sp.		x	Atrichopogon ZUR-51
					n. sp.		x	Atrichopogon ZUR-52
					n. sp.		x	Atrichopogon ZUR-53
					n. sp.		x	Atrichopogon ZUR-54
					n. sp.		x	Atrichopogon ZUR-56
					n. sp.		x	Atrichopogon ZUR-58
		Forcipomyia	*F. (Caloforcipomyia)*	*F. glauca*		x	x	Forcipomyia ZUR-60
			F. (Caloforcipomyia)	*F. hermosa*			x	Forcipomyia ZUR-61
			F. (*Caloforcipomyia*)	*F. sabalitensis*		x	x	Forcipomyia ZUR-1
			F. (Caloforcipomyia)		n. sp.	x		Forcipomyia ZUR-17
			F. (Euprojoannisia)		n. sp.	x	x	Forcipomyia ZUR-15
			F. (Euprojoannisia)		n. sp.	x	x	Forcipomyia ZUR-18
			F. (Euprojoannisia)		n. sp.	x		Forcipomyia ZUR-19
			F. (Euprojoannisia)		n. sp.	x		Forcipomyia ZUR-4
			F. (Euprojoannisia)		n. sp.	x		Forcipomyia ZUR-54

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TABLE 2. (Continued)

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
			F. (Euprojoannisia)		n. sp.	x	x	Forcipomyia ZUR-56
			F. (Euprojoannisia)		n. sp.	x		Forcipomyia ZUR-57
			F. (Euprojoannisia)		n. sp.		x	Forcipomyia ZUR-67
			F. (Euprojoannisia)		n. sp.	x		Forcipomyia ZUR-9
			F. (Forcipomyia)		n. sp.	x	x	Forcipomyia ZUR-12
			F. (Forcipomyia)		n. sp.	x	x	Forcipomyia ZUR-23
			F. (Forcipomyia)		n. sp.	x		Forcipomyia ZUR-51
			F. (Forcipomyia)		n. sp.	x	x	Forcipomyia ZUR-6
			F. (Forcipomyia)		n. sp.	x		Forcipomyia ZUR-8
			F. (Forcipomyia)(argenteola species group*)*	*F. argenteola*		x		Forcipomyia ZUR-29
			F. (Lasiohelea)	*F. attenuata*		x		Forcipomyia ZUR-47
			F. (Lasiohelea)	*F. cornuta*		x	x	Forcipomyia ZUR-11
			F. (Lasiohelea)	*F. stylifera*		x	x	Forcipomyia ZUR-22
			F. (Lasiohelea)		n. sp.	x		Forcipomyia ZUR-44
			F. (Lasiohelea)		n. sp.	x	x	Forcipomyia ZUR-46
			F. (Lasiohelea)		n. sp.	x		Forcipomyia ZUR-48
			F. (Lepidohelea)		n. sp.	x		Forcipomyia ZUR-32
			F. (Lepidohelea)(annulatipes species group*)*	*F. convexipennis*			x	Forcipomyia ZUR-63
			F. (Lepidohelea)(annulatipes species group*)*	*F. annulatipes*		x		Forcipomyia ZUR-31
			F. (Lepidohelea)(annulatipes species group*)*	*F. brasiliensis*		x	x	Forcipomyia ZUR-2
			F. (Lepidohelea)(bicolor species group*)*	*F. dubia*		x		Forcipomyia ZUR-30
			F. (Lepidohelea)(bicolor species group)		n. sp.	x		Forcipomyia ZUR-14
			F. (Lepidohelea)(bicolor species group*)*		n. sp.	x		Forcipomyia ZUR-34
			F. (Lepidohelea)(seminole species group)		n. sp.	x		Forcipomyia ZUR-26

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TABLE 2. (Continued)

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
			F. (Lepidohelea)(seminole species group)	*F. luteigenua*		x		Forcipomyia ZUR-25
			F. (Metaforcipomyia)	*F. albipluma*		x		Forcipomyia ZUR-24
			F. (Metaforcipomyia)	*F. longiflagellata*		x		Forcipomyia ZUR-37
			F. (Metaforcipomyia)	*F. ronderosae*		x		Forcipomyia ZUR-3
			F. (Metaforcipomyia)	*F. rursa*		x	x	Forcipomyia ZUR-41
			F. (Metaforcipomyia)		n. sp.	x	x	Forcipomyia ZUR-20
			F. (Metaforcipomyia)		n. sp.	x		Forcipomyia ZUR-40
			F. (*Microhelea*)		n. sp.	x		Forcipomyia ZUR-10
			F. (Microhelea)		n. sp.	x		Forcipomyia ZUR-27
			F. (Microhelea)		n. sp.	x	x	Forcipomyia ZUR-7
			F. (Pedilohelea)	*F. archboldi*		x	x	Forcipomyia ZUR-35
			F. (Pedilohelea)	*F. spillmani*			x	Forcipomyia ZUR-64
			F. (Rynchoforcipomyia)	*F. dorsalis*		x		Forcipomyia ZUR-52
			F. (Rynchoforcipomyia)		n. sp.	x		Forcipomyia ZUR-5
			F. (Rynchoforcipomyia)		n. sp.	x		Forcipomyia ZUR-53
			F. (Saliohelea)	*F. digita*		x		Forcipomyia ZUR-21
			F. (Saliohelea)	*F. leei*		x	x	Forcipomyia ZUR-16
			F. (Saliohelea)		n. sp.	x	x	Forcipomyia ZUR-45
			F. (Schizoforcipomyia)	*F. harpa*		x		Forcipomyia ZUR-33
			F. (Synthyridomyia)		n. sp.	x		Forcipomyia ZUR-55
			F. (Thyridomyia)	*F. nana*		x		Forcipomyia ZUR-39
			F. (Thyridomyia)	*F. nodosa*			x	Forcipomyia ZUR-65
			F. (Thyridomyia)		n. sp.	x		Forcipomyia ZUR-42
			F. (Thyridomyia)		n. sp.	x		Forcipomyia ZUR-43
			F. (Thyridomyia)		n. sp.	x		Forcipomyia ZUR-49

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TABLE 2. (Continued)

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
			F. (Thyridomyia)		n. sp.	x		Forcipomyia ZUR-50
			F. (Thyridomyia)		n. sp.		x	Forcipomyia ZUR-69
			F. (Warmkea)	*F. lesliei*		x	x	Forcipomyia ZUR-13
Ceratopogoninae	Culicoidini	*Culicoides*	*C. (Anilomyia) (covagarciai* species group)	*C. covagarciai* *		x	x	Culicoides ZUR-1
			C. (Anilomyia) (covagarciai species group)	*C. efferus* *		x	x	Culicoides ZUR-3
			C. (Anilomyia) (covagarciai species group)	*C. metagonatus* *		x	x	Culicoides ZUR-5
			C. (Anilomyia) (covagarciai species group)	*C. monicae* *		x	x	Culicoides ZUR-19
			C. (Anilomyia) (covagarciai species group)		n. sp. *	x	x	Culicoides ZUR-2
			C. (Anilomyia) (decor species group)	*C. chaverri*		x		Culicoides ZUR-24
			C. (Anilomyia) (decor species group)		n. sp.	x		Culicoides ZUR-23
			C. (Culicoides) (luteovenus species group)	*C. elutus* *		x		Culicoides ZUR-6
			C. (Dymodesmyia)		n. sp. *	x		Culicoides ZUR-13
			C. (Glaphiromyia)	*C. scopus* *		x		Culicoides ZUR-7
			C. (Glaphiromyia)		n. sp.	x		Culicoides ZUR-27
			C. (Haematomyidium)	*C. darlingtonae*		x		Culicoides ZUR-17
			C. (Haematomyidium)	*C. neoparaensis*		x		Culicoides ZUR-30
			C. (Hoffmania) (guttatus species group)	*C. annettae* *		x	x	Culicoides ZUR-4
			C. (Hoffmania) (hylas species group)	*C. palpalis*		x		Culicoides ZUR-20
			C. (Hoffmania) (hylas species group)	*C. verecundus* *		x		Culicoides ZUR-12
			daedalus species group	*C. dunni*		x		Culicoides ZUR-14
			daedalus species group	*C. picadoae*		x		Culicoides ZUR-21
			daedalus species group		n. sp. *	x		Culicoides ZUR-15
			daedalus species group	*C. cummingi* *		x	x	Culicoides ZUR-11
			eublepharus species group	*C. rangeli* *		x		Culicoides ZUR-10
			eublepharus species group		n. sp.	x	x	Culicoides ZUR-18

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TABLE 2. (Continued)

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
			eublepharus species group		n. sp.	x		Culicoides ZUR-26
			fluvialis species group	*C. tetrathyris*		x		Culicoides ZUR-28
			monticola species group	*C. monticola*		x		Culicoides ZUR-8
			monticola species group		n. sp. *	x		Culicoides ZUR-16
			monticola species group		n. sp. *	x		Culicoides ZUR-9
			venezuelensis species group	*C. venezuelensis*		x	x	Culicoides ZUR-25
	Ceratopogonini	*Alluaudomyia*		*A. tenuiannulata*		x		Alluaudomyia ZUR-1
		Baeohelea		*B. nana*		x	x	Baeohelea ZUR-1
					n. sp.	x	x	Baeohelea ZUR-2
					n. sp.	x	x	Baeohelea ZUR-3
		Brachypogon	*B.* (*Brachypogon*)		n. sp.	x	x	Brachypogon ZUR-3
			B. (*Brachypogon*)		n. sp.	x	x	Brachypogon ZUR-4
			B. (*Brachypogon*)		n. sp.		x	Brachypogon ZUR-6
			B. (*Brachypogon*)		n. sp.		x	Brachypogon ZUR-7
			B. (*Isohelea*)		n. sp.	x	x	Brachypogon ZUR-1
			B. (*Isohelea*)		n. sp.	x	x	Brachypogon ZUR-2
			B. (*Isohelea*)		n. sp.		x	Brachypogon ZUR-5
		Downeshelea		*D. jurgeni*		x	x	Downeshelea ZUR-1
				D. magna			x	Downeshelea ZUR-3
				D. moravia		x		Downeshelea ZUR-2
		Monohelea		*M. brasiliensis*		x		Monohelea ZUR-1
					n. sp.	x	x	Monohelea ZUR-2
					n. sp.	x		Monohelea ZUR-3
					n. sp.		x	Monohelea ZUR-4
					n. sp.		x	Monohelea ZUR-5
					n. sp.		x	Monohelea ZUR-6

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TABLE 2. (Continued)

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
		Schizonyxhelea			n. sp.	x	x	Schizonyxhelea ZUR-1
					n. sp.		x	Schizonyxhelea ZUR-2
					n. sp.		x	Schizonyxhelea ZUR-3
		Stilobezzia	*S*. (*Acanthohelea*)		n. sp.	x		Stilobezzia ZUR-1
			S. (*Acanthohelea*)		n. sp.	x		Stilobezzia ZUR-2
			S. (*Acanthohelea*)		n. sp.	x	x	Stilobezzia ZUR-3
			S. (*Acanthohelea*)		n. sp.	x		Stilobezzia ZUR-4
			S. (*Acanthohelea*)		n. sp.	x		Stilobezzia ZUR-6
			S. (*Acanthohelea*)		n. sp.		x	Stilobezzia ZUR-10
			S. (*Stilobezzia*)		n. sp.	x		Stilobezzia ZUR-5
			S. (*Stilobezzia*)		n. sp.		x	Stilobezzia ZUR-7
			S. (*Stilobezzia*)		n. sp.		x	Stilobezzia ZUR-9
	Parabezziini	*Parabezzia*			n. sp.	x		Parabezzia ZUR-1
					n. sp.		x	Parabezzia ZUR-2
		Spinellihelea			n. sp.	x	x	Spinellihelea ZUR-1
	Echinoheleini	*Echinohelea*			n. sp.	x	x	Echinohelea
	Heteromyiini	*Clinohelea*			n. sp.	x	x	Clinohelea ZUR-1
					n. sp.	x	x	Clinohelea ZUR-2
	Palpomyiini	*Amerohelea*			n. sp.		x	Amerohelea ZUR-1
		Bezzia	*B*. (*Bezzia*) (*gibbera* species group)		n. sp.	x	x	Bezzia ZUR-1
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x		Bezzia ZUR-10
			B. (*Bezzia*) (*gibbera* species group)		n. sp.		x	Bezzia ZUR-11
			B. (*Bezzia*) (*gibbera* species group)		n. sp.		x	Bezzia ZUR-12
			B. (*Bezzia*) (*gibbera* species group)		n. sp.		x	Bezzia ZUR-13
			B. (*Bezzia*) (*gibbera* species group)		n. sp.		x	Bezzia ZUR-14

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TABLE 2. (Continued)

Subfamily	Tribe	Genus	Subgenus and/or species group	Named (* = barcoded)	new species	Zurquí	Tapantí	ZADBI code
			B. (*Bezzia*) (*gibbera* species group)	*B. pseudogibbera*			x	Bezzia ZUR-15
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x	x	Bezzia ZUR-2
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x		Bezzia ZUR-3
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x		Bezzia ZUR-3A
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x		Bezzia ZUR-4
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x	x	Bezzia ZUR-5
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x		Bezzia ZUR-6
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x	x	Bezzia ZUR-7
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x		Bezzia ZUR-8
			B. (*Bezzia*) (*gibbera* species group)		n. sp.	x		Bezzia ZUR-9
		Palpomyia			n. sp.	x	x	Palpomyia ZUR-1
					n. sp.	x	x	Palpomyia ZUR-2
			distincta species group	*P. paulistensis*		x	x	Palpomyia ZUR-3
					n. sp.	x	x	Palpomyia ZUR-4
					n. sp.	x	x	Palpomyia ZUR-5
					n. sp.	x		Palpomyia ZUR-6
					n. sp.	x	x	Palpomyia ZUR-7
					n. sp.	x	x	Palpomyia ZUR-8
					n. sp.		x	Palpomyia ZUR-10
				P. crassicrus			x	Palpomyia ZUR-11
					n. sp.		x	Palpomyia ZUR-12
	Stenoxenini	*Stenoxenus*		*S. arcuatus*			x	Stenoxenus ZUR-1

The barcoded specimens reported from elsewhere in Costa Rica, primarily from the ACG, were part of a large survey of insects (Janzen & Hallwachs 2019), with records on BOLD (Ratnasingham *et al*. 2024). These records also included a species proxy, the barcode index number (BIN). BINs are an online framework that clusters barcode sequences algorithmically (Ratnasingham & Hebert, 2013). The Ceratopogonidae were fully extracted in Guelph, Ontario, Canada from Malaise trap samples collected from the sites noted below and general protocols are described by Hebert *et al*. (2018) and Janzen *et al*. (2020). ACG traps were from four locations and are shown on the map in figure 2A, with acronyms used in a variety of studies (Janzen *et al.* 2020, Sharkey *et al.* 2020). They are utilized here as follows, giving the dates of collection, elevation and general habitat:

Bosque San Emilio (BSE), Guanacaste Province—10.84389N 85.61384W, March 19, 2012–March 18, 2013, July 11, 2019–July 9, 2020, one trap; 100-year old, 10–20 m tall, secondary successional Pacific dry forest in Sector Santa Rosa of Área de Conservación Guanacaste, 300 m.

San Gerardo (ESG), Alajuela Province—10.88009N 85.38887W, Aug. 19, 2013–Aug. 17, 2015, one trap; 30–80-year old mid-elevation Caribbean rain forest in Sector San Cristobal of Área de Conservación Guanacaste, 575 m.

PL12—Geothermal development platform PL12, Guanacaste Province, 10.76N 85.33W, Nov. 14, 2013–Nov. 5, 2020, nine traps; area of ca. 3 km^2 of forest and edge mostly composed of old growth, mixed dry and rain forest of the Área de Conservación Guanacaste, 791–853 m.

Derrumbe, Guanacaste Province—10.92918N 85.46426W, Oct. 23, 2014–Oct. 20. 2016, one trap; trailside in a somewhat fragmented old-growth cloud forest near the top of 1500 m Volcán Cacao, a member of Cordillera Guanacaste separating BSE from ESG, of the Área de Conservación Guanacaste, 1220 m.

A specimen from each of the ACG BINs was identified to genus by AB, GRS and FD from low resolution photographs provided through BOLD. The rarefaction curve of the ACG BINs was produced with the R package iNEXT (Hsieh *et al*. 2024). Further BINs from Costa Rica reported here were sampled with Malaise traps from a variety of locations (Fig. 2B) and represent a total of 84 further Malaise trap years of sampling and an additional 152,494 barcoded specimens.

Specimens from Refugio los Volcanes, Bolivia were examined only in alcohol and the estimate of the number of species from there is therefore almost certainly an underestimate that would be significantly improved with slide mounting, planned for the future.

Acknowledgments

This paper is partially a result of the large biodiversity project undertaken to interpret the entire Diptera fauna at Zurquí and part of that at Tapantí and Las Alturas in Costa Rica (Borkent *et al.* 2018, Brown *et al.* 2018). As such, the first author gives his profound thanks to his co-PI Brian Brown who shared in the organization and massive logistical tasks involved. That project was supported by National Science Foundation (USA) grant DEB 1145890 to Brian V. Brown and Art Borkent. We thank Instituto Nacional de Biodiversidad, now under the auspices of the Museo Nacional de Costa Rica, for providing laboratory facilities and logistical assistance. The following Costa Rican technicians collected, sorted, and curated the vast bulk of samples with tremendous skill and commitment: Carolina Avila, Marco Moraga, Annia Picado, Wendy Porras, Elena Ulate, and Elvia Zumbado. Annia Picado slide mounted the vast majority of the slides of Ceratopogonidae from the ZADBI project with her remarkable and meticulous skills and identified the majority to genus. Our two program managers at the Los Angeles County Museum, California, Anna Holden and Estella Hernandez, helped to organize the logistics of our project. We extend our deepest appreciation to Jorge Arturo Lizano for his generous permission to work on his property at Zurquí de Moravia for a one year period.

The first author thanks his wife, travelling companion and closest friend Annette Borkent for providing moral and financial support throughout the writing of this contribution and for her steadfast faith in his work, including the years of the ZADBI project. She also shared in the adventure of living in Bolivia for three months in 2016–2017 and assisting with the collecting of biting midges there. We appreciated the advice regarding Costa Rican habitats and literature by Paul E. Hanson, Simuliidae by Peter H. Adler, Chironomidae by John H Epler and Odonata by Robert A. Cannings. We thank the S.W. Williston Diptera Research Fund for partial but significant financial support for the collecting expedition to Bolivia. Albert Schwiening kindly gave generous support and permission to collect at his beautiful reserve at Refugio los Volcanes, Bolivia. We also thank Fabiana Mendez Raya, Directora Ejecutiva, Museo Nacional de Historia Natural, Bolivia for arranging collecting permits while in that country. We thank Margarita Roca for help with some of the graphics.

All specimens that were DNA barcoded from Costa Rica in BOLD were exported and DNA barcoded under Costa Rican government permits issued to BioAlfa (Janzen and Hallwachs 2019) (R-054-2022-OT-CONAGEBIO; R-019-2019-CONAGEBIO; National Published Decree #41767), JICA-SAPI #0328497 (2014) and DHJ and WH (ACG-PI-036-2013; R-SINAC-ACG-PI-061-2021; Resolución Nº001-2004 SINAC; PI-028-2021). We thank the parataxonomist team of the ACG for running the ACG malaise traps, and Roberto Fernandez, Jenny Phillips and Annia Picado for handling their shipping to Guelph, Ontario, Canada for them, and adding/processing those in the BioAlfa Malaise traps from other parts of Costa Rica to be DNA barcoded. BioAlfa was a bioliteracy and DNA barcoding program supported by the Costa Rican government (Janzen & Hallwachs 2019).

We express our thanks to Ryszard Szadziewski and one anonymous person for their reviews of this paper.

Results

How Many Named and Unnamed Species of Biting Midges are present in Costa Rica, with Comparisons to Localities Elsewhere?

The ZADBI project initially recorded 200 species of Ceratopogonidae from the cloud forest at Zurquí, with its multiple traps, and 130 from Tapantí, with a single Malaise trap (Borkent *et al.* 2018, Brown *et al.* 2018). Since then, we have refined our examination of the material and recognize that some morphotypes were recorded twice and a few were too damaged to include in the present study. As such, we currently identify 192 species of Ceratopogonidae from Zurquí and 124 from Tapantí (Tables 2, 3). Of the species at Zurquí only 49 (25.5%) are named and of those at Tapantí 30 (24.2%) are named. Together these two cloud forest sites, separated by only 40 kms, produced 245 species with only 23.3% being named. Of this combined total, 57 were previously named and 188 recognized as new and needing description and names. This level of unnamed diversity is remarkable when compared to the total number of 430 named species known from all of Costa Rica and 1,314 species for the entire Neotropical Region. If 192 species can be sampled from a mere four hectares of cloud forest in Costa Rica, equal to 45% of named Costa Rican, 15% of those in the Neotropical Region, and 3% of the world's named species it is clear that there remains a huge fauna yet to be discovered and described.

Understanding the degree of endemism present is vital to interpreting diversity levels and we have limited information in this regard. Zurquí had 92 endemic species, Tapantí had 46 and together, they had a total of 190 endemic species (Table 4). This almost certainly reflects, to a certain degree, the lack of collecting and taxonomic study. However, comparison of the faunas at Zurquí and Tapantí (Table 3, Fig. 3A) shows that of a total of 245 species present at the two sites, only 73 (30%) were shared between them, suggesting a high-level faunal turnover. Another bias, however, is that this is a comparison between the results of multiple trapping at Zurquí and a single Malaise trap from Tapantí, certainly skewing the results. Comparison of only Malaise trap #1 at Zurquí (that on the forest edge) with that at Tapantí (also on the forest edge) indicates that of a combined total of 186 species, 49 (26%) were shared (Fig. 3B). Malaise trap #2 at Zurquí (located in a ravine) compared with that at Tapantí produced a total of 141 species with 26 (18%) shared between them (Fig. 3C). This too suggests significant local endemism.

Of the 17 species of Ceratopogonidae sampled at more distant Las Alturas, 9 were shared with both Zurquí and Tapantí, none with just Tapantí and 2 with just Zurquí (bypassing Tapantí geographically). Finding 6 of 17 species only in Las Alturas also suggests that the cloud forests of Costa Rica and other Neotropical countries are highly species-rich certainly a pattern found in many other groups. In addition, these six endemic species were represented by only one specimen each, other than one *Palpomyia* with two specimens.

TABLE 3. Number of named and unnamed species of each genus from Zurquí and Tapantí, Costa Rica and their comparative distributions between these two sites and more broadly. The number of named species from Costa Rica and the Neotropical Region are also listed for genera not present at Zurquí and Tapantí. Subfamilies and tribes are in phyletic sequence.

Subfamily	Tribe	Genus	Zurquí # species	named	n. sp.	Tapantí # species	named	n. sp.	Total # species in Zurquí and Tapantí	# named species for total	# n. sp. for total	% n. sp.	# spp. shared between Zurquí and Tapantí	% of total species shared between Zurquí and Tapantí	# named species in Costa Rica + Panama, incl. named from Z+T	# named species in Neotropics	% spp. in Z+T (inc. n.sp.) / named Neotropics	# spp. in Zurquí MT #1	# spp. in Zurquí MT #2
Forcipomyiinae	Dasyheleini	*Dasyhelea*	22	2	20	16	1	15	31	2	29	93.5%	7	22.58%	17	104	29.8%	12	2
	Forcipomyiini	*Atrichopogon*	43	1	42	27	1	26	58	2	56	96.6%	12	20.69%	30	120	48.3%	23	8
		Forcipomyia	55	21	34	25	13	12	61	25	36	59.0%	19	31.15%	85	235	26.0%	37	11
Ceratopogoninae	Culicoidini	*Culicoides*	28	19	9	9	8	2	28	19	9	32.1%	9	32.14%	128	302	11.9%	17	5
	Ceratopogonini	*Alluaudomyia*	1	1	0	0	0	0	1	1	0	0.0%	0	0.00%	4	18	5.6%	0	0
		Baeohelea	3	1	2	3	1	2	3	1	2	66.7%	3	100.00%	1	1	300.0%	3	3
		Brachypogon	4	0	4	7	0	7	7	0	7	100.0%	4	57.14%	6	30	23.3%	2	3
		Downeshelea	2	2	0	2	2	0	3	3	0	0.0%	1	33.33%	24	35	8.6%	1	1
		Monohelea	3	1	2	4	0	4	6	0	6	100.0%	1	16.67%	10	27	22.2%	1	2
		Schizonyxhelea	1	0	1	3	0	3	3	1	2	66.7%	1	33.33%	7	8	37.5%	1	0
		Stilobezzia	6	0	6	4	0	4	9	0	9	100.0%	1	11.11%	30	92	9.8%	1	0
	Parabezziini	*Parabezzia*	1	0	1	1	0	1	2	0	2	100.0%	0	0.00%	8	27	7.4%	0	0
		Spinellihelea	1	0	1	1	0	1	1	0	1	100.0%	1	100.00%	1	1	100.0%	0	0
	Echinoheleini	*Echinohelea*	1	0	1	1	0	1	1	0	1	100.0%	1	100.00%	5	11	9.1%	1	1
	Heteromyiini	*Clinohelea*	2	0	2	2	0	2	2	0	2	100.0%	2	100.00%	5	11	18.2%	1	1
	Palpomyiini	*Amerohelea*	0	0	0	1	0	1	1	0	1	100.0%	0	0.00%	4	13	7.7%	0	0
		Bezzia	11	0	11	8	1	8	16	1	15	93.8%	4	25.00%	20	49	32.7%	6	3
		Palpomyia	8	1	7	9	2	7	11	2	9	81.8%	7	63.64%	5	54	20.4%	5	4
	Stenoxenini	*Stenoxenus*	0	0	0	1	1	0	1	1	0	0.0%	0	0.00%	6	16	6.3%	0	0
		Total	192	49	143	124	30	96	245	58	187		73	29.80%	396	1154	21.2%	111	44
		% named	25.52%			24.19%			23.67%										

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TABLE 3. (Continued)

Subfamily	Tribe	Genus	Zurquí # species	named	n. sp.	Tapantí # species	named	n. sp.	Total # species in Zurquí and Tapantí	# named species for total	# n. sp. for total	% n. sp.	# spp. shared between Zurquí and Tapantí	% of total species shared between Zurquí and Tapantí	# named species in Costa Rica + Panama, incl. named from Z+T	# named species in Neotropics	% spp. in Z+T (inc. n.sp.) / named Neotropics	# spp. in Zurquí MT #1	# spp. in Zurquí MT #2
Neotropical genera not in Zurquí or Tapantí																			
Leptoconopinae		*Leptoconops*													4	13			
Ceratopogoninae	Culicoidini	*Paradasyhelea*													0	3			
	Ceratopogonini	*Allohelea*													1	1			
		Austrohelea													0	3			
		Baeodasymyia													3	5			
		Bahiahelea													0	1			
		Borkenthelea													0	4			
		Cacaohelea													4	4			
		Ceratoculicoides													0	1			
		Isthmohelea													0	1			
		Leptohelea													1	1			
		Macrurohelea													0	17			
		Nannohelea													0	1			
		Notiohelea													0	2			
		Parastilobezzia													1	1			
		Rhynchohelea													1	1			
	Parabezziini	*Diaphanobezzia*													0	4			
		Fittkauhelea													0	1			
		Yungahelea													0	1			
	Heteromyiini	*Heteromyia*													2	11			

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TABLE 3. (Continued)

Subfamily	Tribe	Genus	Zurquí # species	named	n. sp.	Tapantí # species	named	n. sp.	Total # species in Zurquí and Tapantí	# named species for total	# n. sp. for total	% n. sp.	# spp. shared between Zurquí and Tapantí	% of total species shared between Zurquí and Tapantí	# named species in Costa Rica + Panama, incl. named from Z+T	# named species in Neotropics	% spp. in Z+T (inc. n.sp.) / named Neotropics	# spp. in Zurquí MT #1	# spp. in Zurquí MT #2
		Pellucidomyia													2	4			
		Physohelea													0	2			
	Johannsenomyiini	*Austrosphaeromias*													0	4			
		Groganhelea													0	1			
		Johannsenomyia													1	2			
		Lanehelea													0	2			
		Mallochohelea													1	6			
		Neobezzia													4	8			
		Nilobezzia													1	3			
		Sphaerohelea													0	1			
	Palpomyiini	*Clastrieromyia*													0	4			
		Pachyhelea													1	2			
		Phaenobezzia													1	2			
	Stenoxenini	*Paryphoconus*													6	43			
		Total # named spp													430	1314			

TABLE 4. Distributions of named and unnamed species from Zurquí and Tapantí, Costa Rica. Subfamilies and tribes are in phyletic sequence.

Subfamily	**Tribe**	**Genus**	**endemic to Zurquí**	**endemic to Tapantí**	**endemic to Zurquí + Tapantí**	**Total endemic for either/ both Zurquí, Tapantí**	**others from Zurquí + Tapantí also endemic to Costa Rica**	**total from Zurquí + Tapantí endemic to Costa Rica**	**Zurquí and/or Tapantí distributed northward**	**Zurquí and/ or Tapantí distributed southward**	**Zurquí and/ or Tapantí broadly distributed**
Forcipomyiinae	Dasyheleini	*Dasyhelea*	14	9	6	29	0	29	0	2	0
	Forcipomyiini	*Atrichopogon*	30	15	12	57	2	59	0	0	0
		Forcipomyia	24	2	10	36	6	42	2	5	12
Ceratopogoninae	Culicoidini	*Culicoides*	7	0	2	9	5	14	0	5	9
	Ceratopogonini	*Alluaudomyia*	0	0	0	0	0	0	1	0	0
		Baeohelea	0	0	2	2	0	2	0	1	0
		Brachypogon	0	3	4	7	0	7	0	0	0
		Downeshelea	1	1	0	2	1	3	0	0	0
		Monohelea	1	3	1	5	0	5	0	1	0
		Schizonyxhelea	0	2	1	3	0	3	0	0	0
		Stilobezzia	5	3	1	9	0	9	0	0	0
	Parabezziini	*Parabezzia*	1	1	0	2	0	2	0	0	0
		Spinellihelea	0	0	1	1	0	1	0	0	0
	Echinoheleini	*Echinohelea*	0	0	1	1	0	1	0	0	0
	Heteromyiini	*Clinohelea*	0	0	2	2	0	2	0	0	0
	Palpomyiini	*Amerohelea*	0	1	0	1	0	1	0	0	0
		Bezzia	7	4	4	15	0	15	0	0	1
		Palpomyia	2	2	5	9	0	9	0	2	0
	Stenoxenini	*Stenoxenus*	0	0	0	0	0	0	0	1	0
Total			92	46	52	190	14	204	3	17	22

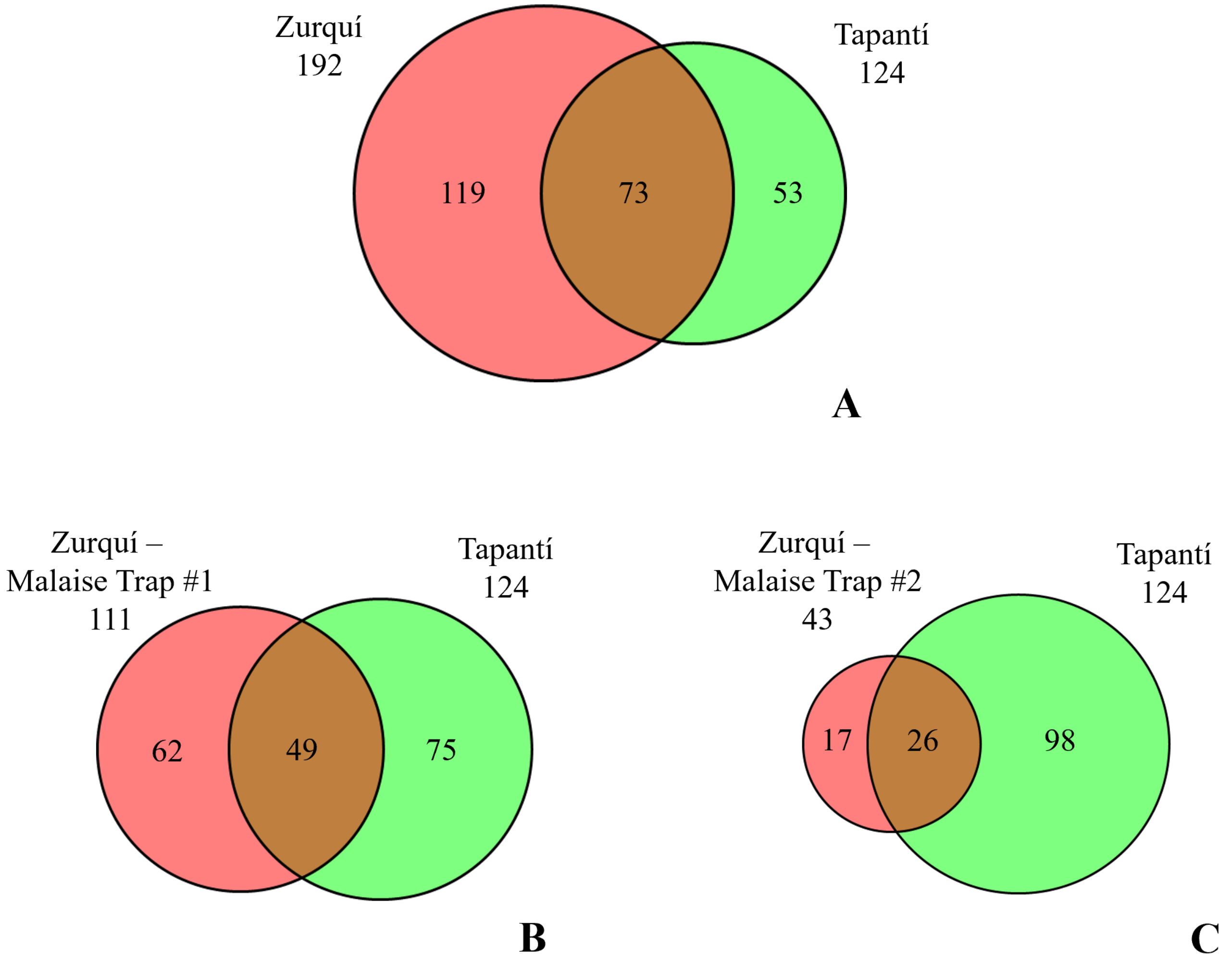

FIGURE 3. Comparison of number of species sampled from A. All traps at Zurquí and Tapantí, Costa Rica. B. Malaise trap #1 at Zurquí and Tapantí, Costa Rica. C. Malaise trap #2 at Zurquí and Tapantí, Costa Rica.

The four genera with the largest number of species at Zurquí and Tapantí (Table 3) are *Dasyhelea*, *Atrichopogon*, *Forcipomyia* and *Culicoides* and the relative numbers of unnamed species in each of these genera (93.5%, 96.6%, 59.0%, 32.1% respectively) reflects the relative taxonomic attention they have been given in the Neotropical Region (Borkent & Spinelli 2007) (and elsewhere). *Culicoides*, with 1,360 species worldwide (Borkent & Dominiak 2020, Borkent *et al.* 2022) is nearly unique among genera of Ceratopogonidae in having females which bite vertebrates and with numbers of species acting as vectors of various pathogens and other organisms, naturally drawing the most attention from taxonomists and other biologists. The only other genera with females feeding on vertebrate blood are *Leptoconops* Skuse (155 spp.), *Austroconops* Wirth & Lee (2 spp.) and *Forcipomyia* (*Lasiohelea* Kieffer) (179 spp.). Both *Dasyhelea* and *Atrichopogon* are the most neglected of these big genera with many species remaining to be described nearly everywhere on the planet (pers. obs.). Some of the genera at Zurquí and Tapantí have more species (named and unnamed) than are named in all of Costa Rica and Panama: *Dasyhelea*, *Atrichopogon*, *Brachypogon* Kieffer and *Palpomyia* (Table 3; Fig. 4). Others are still striking in their diversity: for example, six species of *Monohelea* Kieffer and 16 of *Bezzia* Kieffer at Zurquí and Tapantí compared to 10 and 20 respectively named for all of Costa Rica and Panama. *Dasyhelea*, *Atrichopogon* and *Palpomyia* each have significantly more morphospecies at Zurquí and Tapantí than are named for all of Costa Rica and Panama (Fig. 4). One genus, *Downeshelea* Wirth & Grogan, is unique in our study in that it has all three of the discovered species named (Table 3), while there are 24 named species in Costa Rica and Panama, reflecting the recent revisions by Santarém *et al.* (2018, 2020). These numbers also reflect how poorly known are the species of Costa Rica and the Neotropical Region.

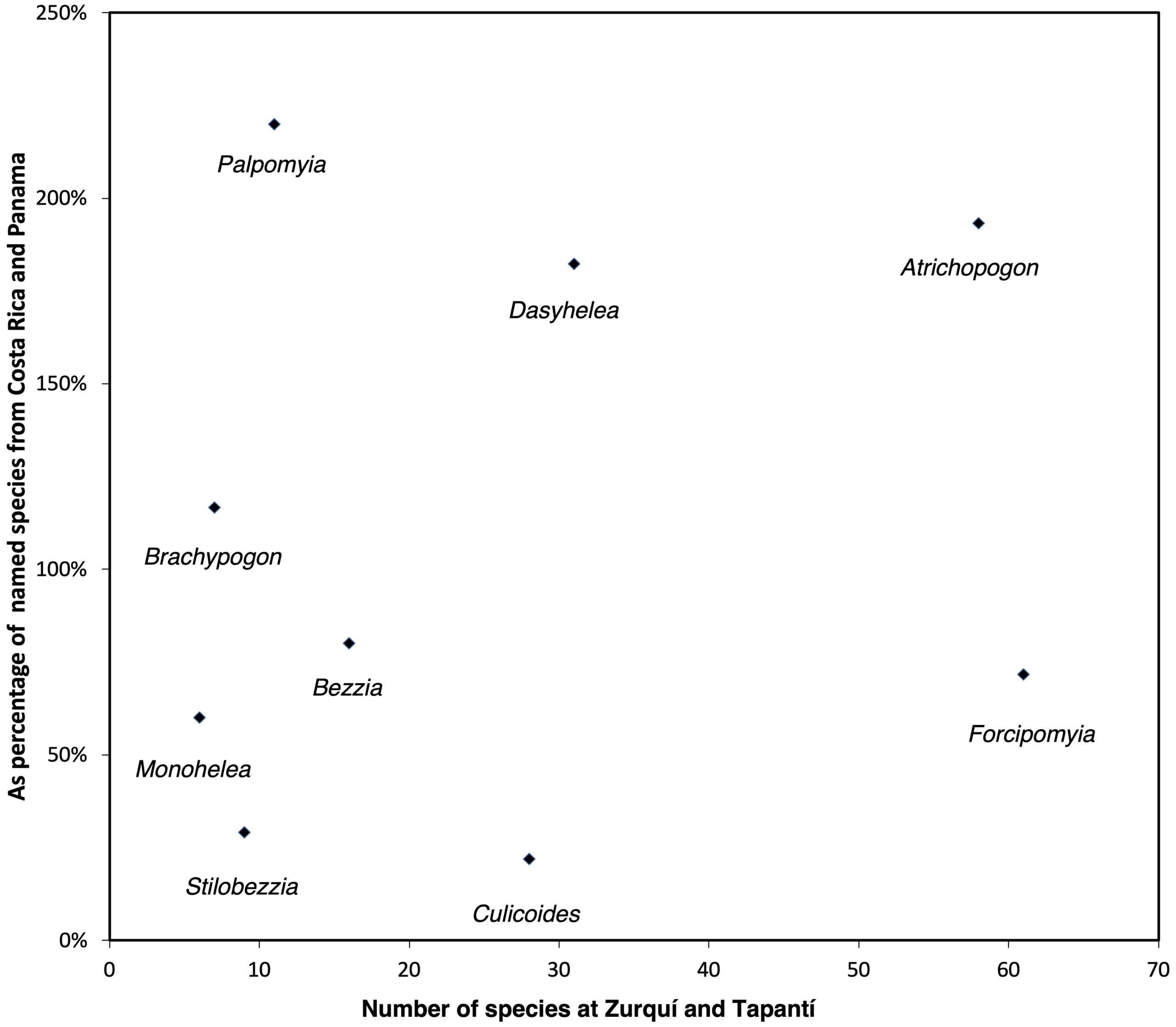

FIGURE 4. Number of named and unnamed species in the cloudforest at Zurquí and Tapantí, Costa Rica as a percentage of the number of named species known from Costa Rica and Panama, for those genera with six or more species at Zurquí and Tapantí.

Spinelli & Borkent (2004) reported 148 species of *Culicoides* from or suspected of living in Costa Rica while here we report only 129. The reason for the discrepancy is that in 2004 we included species known only from Colombia or where Colombia was the most northernly known distribution for a given species but these are excluded here for the reasons explained in Materials & Methods.

Barcoding also indicates a large number of unnamed species in Costa Rica. The study in the ACG (Fig. 2A), sampling from 300–1200 meters, from dry to rainforest habitats, barcoded 45,717 Ceratopogonidae representing 1,060 BINs (Fig. 5A), more than double the 430 named species known from Costa Rica (Table 3). A rarefaction analysis of the ACG specimens (Fig. 5B) indicates the number of BINs had not reached an asymptote and extrapolation to 80,000 specimens indicates the presence of 1,300 BINs. Although BINs do not always equal actual species (Hartop *et al.* 2022, Meier *et al.* 2022), these are still striking numbers. The very limited barcoding done on only 15 species of *Culicoides* from Zurquí accurately corresponded to them being morphologically distinct (Table 2). However, while the first analysis of 45 specimens of *C. covagarciai* indicated the specimens were conspecific, further analysis incorporating specimens from elsewhere in Costa Rica indicated the presence of two BINs, slightly less than 2% p-distance apart. Re-examination of the specimens of this species failed to detect any further morphological differences and we therefore regard these BINs as belonging to the same species. Although being only one examined instance, the *C. covagarciai* example shows that using BINs as a proxy for species might lead to an overestimation of species in Ceratopogonidae. Similar cases are known from other nematoceran families such as Chironomidae (Lin *et al*. 2015) and Sciaridae (Heller *et al*. 2016). Nevertheless, algorithmic delimitation of genetic lineages is our best proxy for species when morphological examination is out of reach, and undoubtedly improves our knowledge on diversity and enables comparisons of communities in previously unchartered taxonomic groups (Hartop *et al*. 2024).

Comparison of the four trapping sites in the ACG (Fig. 5A) reveals a number of patterns. Keeping in mind that PL12 was sampled with 9 Malaise traps for seven years, compared to the other sites, each with one trap for two years, it might be expected that PL12 had the largest number of BINs with 661 present. The lowest number was present in the BSE dry forest at 300 m, with 171 BINs, almost certainly reflecting the more limited diversity of Ceratopogonidae in more xeric habitats, with its limited and often ephemeral aquatic and semiaquatic habitats. Nevertheless, 48 BINs encompassing 31% of the records were unique to this dry forest. It is known that some species of Ceratopogonidae are restricted to dry habitats. Although our understanding of specific larval habitats is mostly lacking in Costa Rica, in southwestern and southern U.S.A. species of *Dasyhelea*, *Forcipomyia* and *Culicoides* have been reared from cacti (Wirth & Hubert 1960). Wirth & Hubert's (1960) revision of the *Culicoides copiosus* group (= subgenus *C*. (*Drymodesmyia* Vargas)) included 14 species, mostly from the Nearctic region. Of these, 8 were reared from "rotting cactus stems". Other breeding sites that they mentioned for this subgenus were: tree holes, rotten calabash (for *C. jamaicensis* Edwards, present in Costa Rica) and flowers of *Heliconia* (for *C. panamensis* Barbosa, also present in Costa Rica). The other two species of *C*. (*Drymodesmyia*) that are present in Costa Rica are *C*. (*D*.) *pilosus* Wirth & Blanton and *C*. (*D*.) *poikilonotus* Macfie both unknown as immatures (Borkent 2014).

The Derrumbe cloud forest, with 231 BINs (Fig. 5A), was fairly distinctive with only 10 BINs (4%) shared with the dry lowland BSE forest and these 10 BINs also present at 791–853 m. The Derrumbe cloud forest shared 86 BINs (37%) with the traps at 575–853 m (ESG, PL12). ESG and PL12 were at similar elevations (575 m and 791–853 m respectively) and yet shared only 82 BINs, representing 28% and 12% of their BINs respectively. These two sites were situated only 14.2 kms from each other, and this too suggests significant levels of endemism in even mid-elevation sites in Costa Rica.

Students of Ceratopogonidae may be struck by the relatively low number of specimens that were generated by the ACG study. Considering that there was a total of 69 trapping years, the presence of 45,717 indicates an average of only 622 specimens per year. It is otherwise common to have 100 or more specimens per week in Malaise traps in the tropics and sometimes much more. However, the latter are from traps placed on the margins of forest habitats, deriving specimens from an edge effect and clearly sampling significantly more than those deep within forest habitats. The Malaise traps from Bosque San Emilio (BSE), San Gerardo (ESG), Derrumbe, and five of the nine traps around the geothermal development platform (PL12) were deep inside forest habitat and an additional three of the nine PL12 traps were initially inside the forest.

Finally, BOLD records for all 221,407 barcoded, putative Ceratopogonidae (at least the strong majority correctly identified to family) of Costa Rica (Fig. 2B), including those from the ACG discussed above, results in 4,023 BINs. Aside from the Malaise traps from the ACG (Fig. 2A), this total number includes 64 additional Malaise traps from elsewhere in Costa Rica (Fig. 2B). A rarefaction analysis of these (Fig. 5C) indicates that the BIN number has not reached an asymptote and projections for 300,000 samples indicate the presence of 4,402 BINs and 400,000 samples indicate 4,724 BINs. Clearly, this represents only a small proportion of the tremendously variable habitats in Costa Rica, with most of the country not surveyed. Contrasting with 430 named Costa Rican species, 1,314 named Neotropical species and over 6,200 species worldwide, this too strongly suggests a huge undescribed diversity yet awaiting description and interpretation.

It is clear that there is remarkable unnamed diversity elsewhere in the Neotropical Region. In general, the Andes, with the most diverse fauna and flora for so many groups otherwise, is poorly collected for Ceratopogonidae, other than some areas sampled for *Culicoides* and this is particularly true of its eastern slopes, where within the Andean area the highest diversity is recorded for many taxa. The first author spent November–December of 2016 and January, 2017 in Bolivia on the eastern side of the Andes sampling from a variety of habitats. Many specimens remain to be studied from that expedition but on Dec. 17, 2016 sampling at Carrasco National Park (at about 520 m), sweeping an aerial net for five hours resulted in 172 specimens representing a remarkable 70 species (Table 5), with an initial examination indicating nearly all of them undescribed.

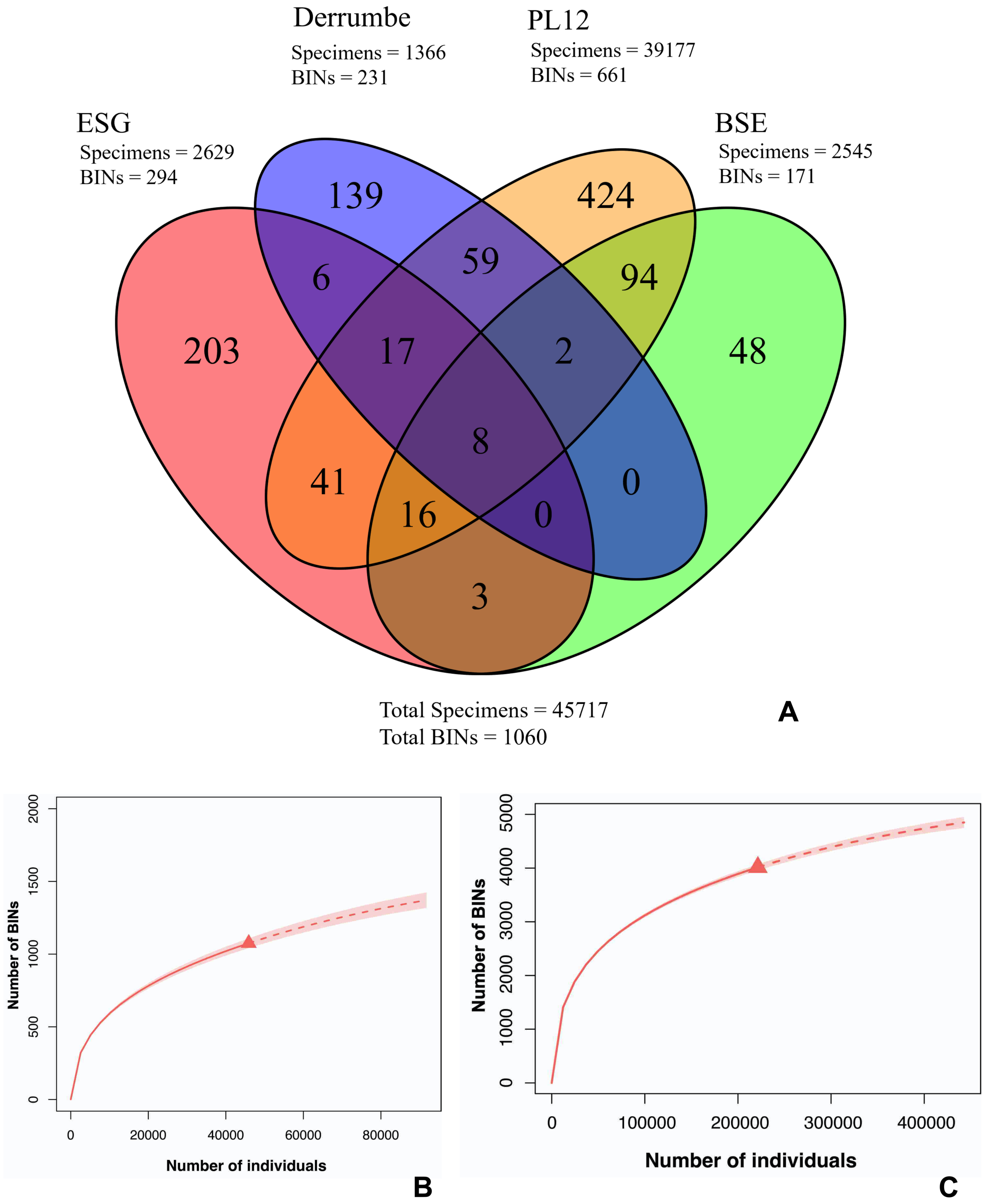

FIGURE 5. A. Venn diagram of number of BINs based on barcoded specimens sampled with Malaise traps from four locations in the Área de Conservación Guanacaste, Costa Rica. Total number of specimens and BINs are indicated for each site as well as the combined numbers from all sites. Site abbreviations, elevations and overall habitat are as follows: BSE = Bosque San Emilio, 300 m, secondary successional Pacific dry forest; ESG = San Gerardo, 575 m, rain forest; PL12 = Geothermal development platform 12 in an area called Pailas II, 791–853 m, old growth, mixed dry and rain forest; Derrumbe, 1220 m, cloud forest. Further details are provided in Materials and Methods. B. Rarefaction curve for 1,060 BINs from the Área de Conservación Guanacaste, Costa Rica. C. Rarefaction curve for 4,023 BINs from all of Costa Rica.

TABLE 5. Comparison of numbers of species from the ZADBI project in Costa with two other Neotropical sites and the United Kingdom, with that from Carrasco NP, Bolivia sampled for 5 hours and that from Refugio Los Volcanes, Bolivia sampled with two Malaise traps for 71 days and 14 days with a sweep net. Subfamilies and tribes are in phyletic sequence.

Subfamily	**Tribe**	**Genus**	**Carrasco NP, Bolivia**	**Refugio Los Volcanes, Bolivia**	**# spp. in Zurquí MT #1**	**Zurquí # species**	**Tapantí # species**	**United Kingdom**
Forcipomyiinae	Dasyheleini	*Dasyhelea*	12	23	12	22	16	14
	Forcipomyiini	*Atrichopogon*	22	34	23	43	27	13
		Forcipomyia	20	34	37	55	25	29
Ceratopogoninae	Culicoidini	*Culicoides*	1	7	17	28	9	47
	Ceratopogonini	*Alluaudomyia*	1		0	1		1
		Baeodasymyia		1				
		Baeohelea			3	3	3	
		Brachypogon	3	1	2	4	7	7
		Allohelea						1
		Ceratopogon						5
		Downeshelea	1	1	1	2	2	
		Kolenohelea						1
		Monohelea			1	3	4	
		Schizohelea						1
		Schizonyxhelea	2	1	1	1	3	
		Serromyia						6
		Stilobezzia	1	8	1	6	4	3

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TABLE 5. (Continued)

Subfamily	Tribe	Genus	Carrasco NP, Bolivia	Refugio Los Volcanes, Bolivia	# spp. in Zurquí MT #1	Zurquí # species	Tapantí # species	United Kingdom
	Parabezziini	*Parabezzia*	1	3	0	1	1	
		Spinellihelea			0	1	1	
	Echinoheleini	*Echinohelea*			1	1	1	
	Heteromyiini	*Clinohelea*	1		1	2	2	1
		Neurohelea						1
	Sphaeromiini	*Sphaeromias*						2
	Johannsenomyiini	*Mallochohelea*	1					4
		Nilobezzia	1					
		Probezzia						2
	Palpomyiini	*Amerohelea*			0	0	1	
		Bezzia	1	3	6	11	8	18
		Phaenobezzia						1
		Palpomyia	2	7	5	8	9	15
	Stenoxenini	*Stenoxenus*		1			1	
		Total	70	124	111	192	124	172

Another site on the eastern side of the Andes in Bolivia was at Refugio Los Volcanes, 5.1 kms NE Bermejo, Dept. Santa Cruz, 18° 6.3'S 63° 35.9'W situated at 1047 m. Two Malaise traps were run there Nov. 13, 2016–Jan. 22, 2017 (i.e. 71 days) as well as intense sweeping of a great number of habitats situated along nearly all the trails and many streams at this reserve for 14 days: Nov. 11–15, 2016, Dec. 27–31, 2016 and Jan. 20–23, 2017. Results from there indicate the presence of at least 124 species (determined in alcohol) (Table 5), compared to the 192 collected at Zurquí, with its many traps for one year, 111 species from Malaise trap #1 at Zurquí and 124 species at Tapantí with one Malaise trap for one year.

Santarém & Felippe-Bauer (2024) reported only 529 described species for all of Brazil but sampling is extremely incomplete. Of 26 states in Brazil, 22 have less than 100 species recorded. Although the size of these states differ substantial, the state of Tocantins with an area of 277,620 km² has no records of Ceratopogonidae at all. The state of Amazonas with an area of 1.571 million km² has only 165 species known, less than that recorded at Zurquí. Clearly, there remains a very large fauna yet to be sampled.

Comparisons with temperate regions shows that the Ceratopogonidae fauna of the Neotropical Region, at least, is far more diverse. The Nearctic fauna has 641 named species of Ceratopogonidae (including Nearctic Mexico) and the Palaearctic has 1,498 (Borkent & Dominiak 2020). In both these regions there are significantly more species awaiting description, especially in the genera *Dasyhelea*, *Atrichopogon*, *Forcipomyia* and *Brachypogon* (although most genera are in need of revision). The British fauna has certainly the best-known fauna in the world and the entire United Kingdom has 172 species of Ceratopogonidae (Chandler 2023) (Table 5). A handbook currently in preparation by the first author and Patrycja Dominiak and based on a dated manuscript by the late John Boorman confirms this number or perhaps slightly more. As such, Zurquí with 194 species sampled from four hectares has more species than all of the UK, with an area of about 244,000 km². The single Malaise trap at Tapantí run for one year sampled 124 species (Table 5). Similarly, the numbers of species in the most diverse European countries (in descending order) have somewhat more to less species than are present at Zurquí (with 192) but nearly equal or less than the combined ZADBI species with 245 species: Germany (256), France (241), Poland (220), Estonia (184), Spain (164), Belgium (151), Ukraine (143), Slovakia (137), Hungary (129), Lithuania (129) (Szadziewski *et al.* 2013; numbers slightly dated). The relative numbers in each of these countries is obviously partially a reflection of differences in their size, topography and location but also reflects where most ceratopogonid taxonomists have worked. Regardless, this too indicates that the fauna of Costa Rica and the Neotropical Region is vastly larger.

The fauna of the Afrotropical Region, with 928 species, seems somewhat depauperate compared to that of the Neotropical Region, as is commonly the case with other taxa. In part this may be due to the radical changes the rain forests of Africa have experienced over time and particularly during the Neogene, shrinking significantly to the present time (Kirk-Spriggs & Muller 2017). South Africa has the most diverse fauna, reflecting its remarkable ecological and geographical diversity. The montane areas are much more limited than in the Neotropics and this too likely has resulted in a more depauperate fauna. Regardless, there is much to be explored throughout the Afrotropical Region.

The Oriental Region, with 1,328 species has nearly exactly the same number of named species as in the Neotropics, with 1,314. The geographical complexity and discontinuous land mass in this Region, with so much of it not yet sampled for Ceratopogonidae provides enticing possibilities for exploration.

There are, unfortunately, no published estimates of numbers of unnamed species from these other Regions. The estimate by Borkent (2017) of at least 9,000 unnamed species of Ceratopogonidae remains, in large measure, a minimum approximation.

Zoogeographic Affinities of ZADBI Species of Biting Midges

There have been very few studies of the impact of elevation on the diversity of Ceratopogonidae, reflecting in general their poorly explored zoogeography. Wirth & Blanton (1959) discussed the distribution of species of *Culicoides* from Panama, noting that some species were restricted to higher elevations, including *C. covagarciai* Ortiz, *C. efferus* Fox, *C. metagonatus* Wirth & Blanton and *C. scopus* Root & Hoffman, also present at Zurquí. Wirth & Lee (1967) described nine *Culicoides* species specifically from the Colombian paramo at 2,500–3,250 m and suggested these had more northern affinities than to the lowlands of Colombia; eight of these are endemic to Colombia and one, *C. monticola* Wirth & Lee is distributed from Costa Rica south to Ecuador. Borkent & Grogan (1995) recognized that species of *Ceratopogon* Meigen were restricted to cold habitats in the Holarctic Region, being either arctic, very early spring species or occurring at higher elevation when present in more southern latitudes.

There were 58 previously named species present at Zurquí and Tapantí and therefore with the potential of interpreting their broader distribution (Tables 2, 6). Of these 16 (28%) are endemic to Costa Rica and Panama, three (5%) have more northerly distributions, 17 (29%) have southern distributions and 22 (38%) had more broad distributions both north and south of Costa Rica and Panama. This suggests that there has been significant endemic speciation in Costa Rica and Panama (Barrantes 2009), although phylogenetic study is needed to show this conclusively. In addition, the high elevations south of Costa Rica have had a significant influence in the composition of the cloud forest fauna of Costa Rica but more broadly distributed species are also an important part of the high elevation fauna.

TABLE 6. Named species from Zurquí and Tapantí, Costa Rica and their broader distributions. Underlined species names indicates the possibility of misidentification. The asterisk indicates a species which also occurs 120 kms north of Costa Rica in Nicaragua. Genera are in tribes arranged in phyletic sequence.

Named species in Zurquí and Tapantí	**Distribution**			
	Endemic to Costa Rica	**Known further north**	**Known further south**	**Broadly distributed in Neotropics**
Dasyhelea hamula			x	
Dasyhelea patrycjae			x	
Atrichopogon colossus	x			
Atrichopogon granditibialis	x			
Forcipomyia (Caloforcipomyia) glauca				x
Forcipomyia (Caloforcipomyia) hermosa			x	
Forcipomyia (Caloforcipomyia) sabalitensis				x
Forcipomyia (Forcipomyia) argenteola				x
Forcipomyia (Lasiohelea) attenuata				x
Forcipomyia (Lasiohelea) cornuta				x
Forcipomyia (Lasiohelea) stylifera				x
Forcipomyia (Lepidohelea) convexipennis			x	
Forcipomyia (Lepidohelea) annulatipes				x
Forcipomyia (Lepidohelea) dubia			x	
Forcipomyia (Lepidohelea) luteigenua			x	
Forcipomyia (Lepidohelea) brasiliensis				x
Forcipomyia (Metaforcipomyia) albipluma	x			
Forcipomyia (Metaforcipomyia) longiflagellata	x			
Forcipomyia (Metaforcipomyia) ronderosae	x			
Forcipomyia (Metaforcipomyia) rursa	x			
Forcipomyia (Pedilohelea) archboldi			x	
Forcipomyia (Pedilohelea) spillmani				x
Forcipomyia (Rynchoforcipomyia.) dorsalis		x		
Forcipomyia (Saliohelea) digita	x			
Forcipomyia (Saliohelea) leei				x
Forcipomyia (Schizoforcipomyia) harpa	x			
Forcipomyia (Thyridomyia) nana				x
Forcipomyia (Thyridomyia) nodosa		x		
Forcipomyia (Warmkea) lesliei				x
Culicoides (Anilomyia) covagarciai				x

......*continued on the next page*

TABLE 6. (Continued)

Named species in Zurquí and Tapantí	**Distribution**			
	Endemic to Costa Rica	**Known further north**	**Known further south**	**Broadly distributed in Neotropics**
Culicoides (Anilomyia) chaverri	x			
Culicoides (Anilomyia) efferus				x
Culicoides (Anilomyia) metagonatus			x *	
Culicoides (Anilomyia) monicae	x			
Culicoides (Culicoides) elutus				x
Culicoides (Glaphiromyia) scopus				x
Culicoides (Haematomyidium) darlingtonae			x	
Culicoides (Haematomyidium) neoparaensis				x
Culicoides (Hoffmania) annettae	x			
Culicoides (Hoffmania) palpalis			x	
Culicoides (Hoffmania) verecundus				x
Culicoides cummingi	x			
Culicoides dunni			x	
Culicoides monticola			x	
Culicoides picadoae	x			
Culicoides rangeli				x
Culicoides tetrathyris				x
Culicoides venezuelensis				x
Alluaudomyia tenuiannulata		x		
Baeohelea nana			x	
Downeshelea jurgeni	x			
Downeshelea moravia	x			
Downeshelea magna	x			
Monohelea brasiliensis			x	
Bezzia pseudogibbera				x
Palpomyia crassicrus			x	
Palpomyia paulistensis			x	
Stenoxenus arcuatus			x	
Total	16	3	17	22

This pattern appears to be true for the entire named fauna of Costa Rica and Panama taken as a whole (Table 7). Of these 127 (29%) are endemic to Costa Rica and Panama, 42 (9%) have more northerly distributions, 108 (25%) have southern distributions and 153 (36%) had more broad distributions both north and south of Costa Rica and Panama. There is some bias in this comparison because the 58 named ZADBI cloud forest species are included. If these are excluded, the remaining 372 named species in Costa Rica may include other high elevation species but we did not determine these and there are at least some included in this group. Of this remaining fauna 113 (30%) are endemic to Costa Rica and Panama, 39 (10%) have more northerly distributions, 91 (24%) have southern distributions and 131 (35%) had more broad distributions both north and south of Costa Rica and Panama. This pattern is also very similar to that of the high elevation named fauna at Zurquí and Tapantí and indicates that the geographic affinities of the Costa Rican and Panamanian fauna noted above for the ZADBI species are broadly present for the entire fauna of this area, with about 30% being endemic and with stronger southern than northern affinities. Furthermore, the pattern described here for Ceratopogonidae is a similar expression for much of the fauna and flora (Halffter 1987), in which there are high levels of endemism, strong South American connections and some

Nearctic elements that are also present further south in South America. The analysis here, however, is restricted only to the species distributions. Phylogenetic patterns of the species would provide significant insight if this were available, a serious gap in our understanding.

TABLE 7. Total numbers of named species in Costa Rica and Panama and their broader distributions. The numbers of named species after eliminating those from Zurquí and Tapantí (ZADBI) are also tabulated. Subfamilies and tribes are in phyletic sequence.

Subfamily	**Tribe**	**Genus**	**Distribution**				
			Endemic to Costa Rica and Panama	**Known further north**	**Known further south**	**Broadly distributed in Neotropics**	**# named species in Costa Rica and Panama**
Leptoconopinae		*Leptoconops*	1			3	4
Forcipomyiinae	Dasyheleini	*Dasyhelea*	3	4	6	4	17
	Forcipomyiini	*Atrichopogon*	25		2	3	30
		Forcipomyia	24	2	29	30	85
Ceratopogoninae	Culicoidini	*Culicoides*	26	18	27	57	128
	Ceratopogonini	*Allohelea*				1	1
		Alluaudomyia		1	2	1	4
		Baeodasymyia	3				3
		Baeohelea			1		1
		Brachypogon	1		1	4	6
		Cacaohelea	4				4
		Downeshelea	9	3	6	6	24
		Leptohelea			1		1
		Monohelea	2	2	5	1	10
		Parastilobezzia			1		1
		Rhynchohelea		1			1
		Schizonyxhelea	3		2	2	7
		Stilobezzia	11	2	7	10	30
	Parabezziini	*Parabezzia*	5	1		2	8
		Spinellihelea	1				1
	Echinoheleini	*Echinohelea*			3	2	5
	Heteromyiini	*Clinohelea*			3	2	5
		Heteromyia			2		2
		Pellucidomyia	1			1	2
	Johannsenomyiini	*Johannsenomyia*	1				1
		Mallochohelea				1	1
		Neobezzia	1		2	1	4
		Nilobezzia				1	1
	Palpomyiini	*Pachyhelea*				1	1
		Phaenobezzia				1	1
		Amerohelea		1	2	1	4
		Bezzia	1	5	2	12	20
		Palpomyia			2	3	5
	Stenoxenini	*Paryphoconus*		1	2	3	6
		Stenoxenus	5	1			6
		Total	127	42	108	153	430

......*continued on the next page*

TABLE 7. (Continued)

Subfamily	Tribe	Genus	Distribution				
			Endemic to Costa Rica and Panama	**Known further north**	**Known further south**	**Broadly distributed in Neotropics**	**# named species in Costa Rica and Panama**
Named ZADBI species			16	3	17	22	
Remaining named species			111	39	91	131	
% of total fauna other than those from ZADBI			30%	10%	24%	35%	

Without phylogenetic analysis and no fossils from this region, combined with an often very incomplete understanding of the complete distributions of many species, it is presently impossible to interpret the historical zoogeography of Costa Rican + Panamanian Ceratopogonidae. When studying the sort of detailed information available for such groups as Culicidae (Wilkerson *et al*. 2021) and Anurans (Savage 2002), it is clear that much more research is needed on Ceratopogonidae. For example, the impact of the formation of the isthmian Central American land bridge about three million years ago and the consequential Great American Interchange of the vertebrate fauna (Rich & Rich 1983) may have influenced the pattern of Ceratopogonidae distributions seen today. The high level of endemism, at least, suggests significant levels of isolation in the relatively recent past (i.e. probably within the last few million years) but this is mere conjecture.

Some further differences between high and low elevation Ceratopogonidae in Costa Rica are discussed further below.

The Diversity of Genera and Lineages of Cloud Forest Biting Midges

It is common practice for authors to examine biodiversity by comparing the numbers of species at a given taxonomic rank (e.g. comparing numbers in different genera or of different families). Examination of the number of species in each of the genera of Ceratopogonidae clearly shows what is a general, worldwide pattern (Borkent & Dominiak 2020, Borkent *et al.* 2022; Table 3). The largest genera at Zurquí and Tapantí (Table 3) are those of *Dasyhelea* (n= 31 species), *Atrichopogon* (n= 58), *Forcipomyia* (n= 61) and *Culicoides* (n= 28) and together these make up 72% of the fauna there. *Stilobezzia* (n= 9), *Bezzia* (n= 16) and *Palpomyia* n= (11) were moderately diverse and the remaining genera had 1–7 species each.

It is important to remember, however, that taxonomic categories above the species level are human constructs to help us group species. As such, comparisons of numbers of species in various genera are arbitrary and misleading. With the advent of Hennigian cladistics, phylogenies can provide genealogically accurate portrayals of the relationships between those categories, representing actual lineages and therefore a basis for logical comparisons between bifurcating lineages. As such, the phyletic diversity of species in given habitats and/or areas can be informative regarding the actual nature of their diversification including the underlying bionomic and/or zoogeographic basis for evident patterns.

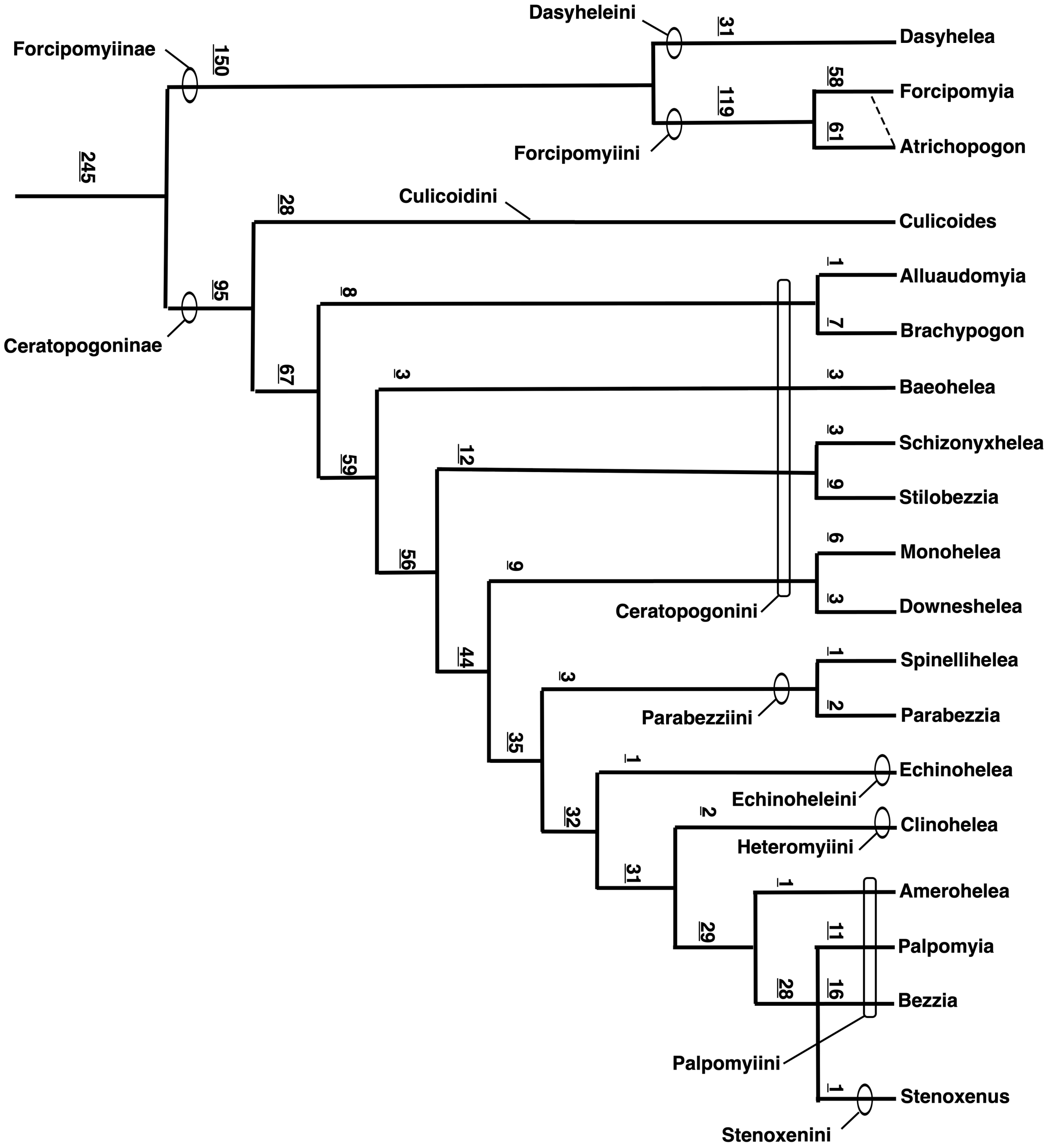


FIGURE 6. Phylogeny of the genera at Zurquí and Tapantí, Costa Rica indicating numbers of species (named and unnamed) of each genus and at each bifurcation.

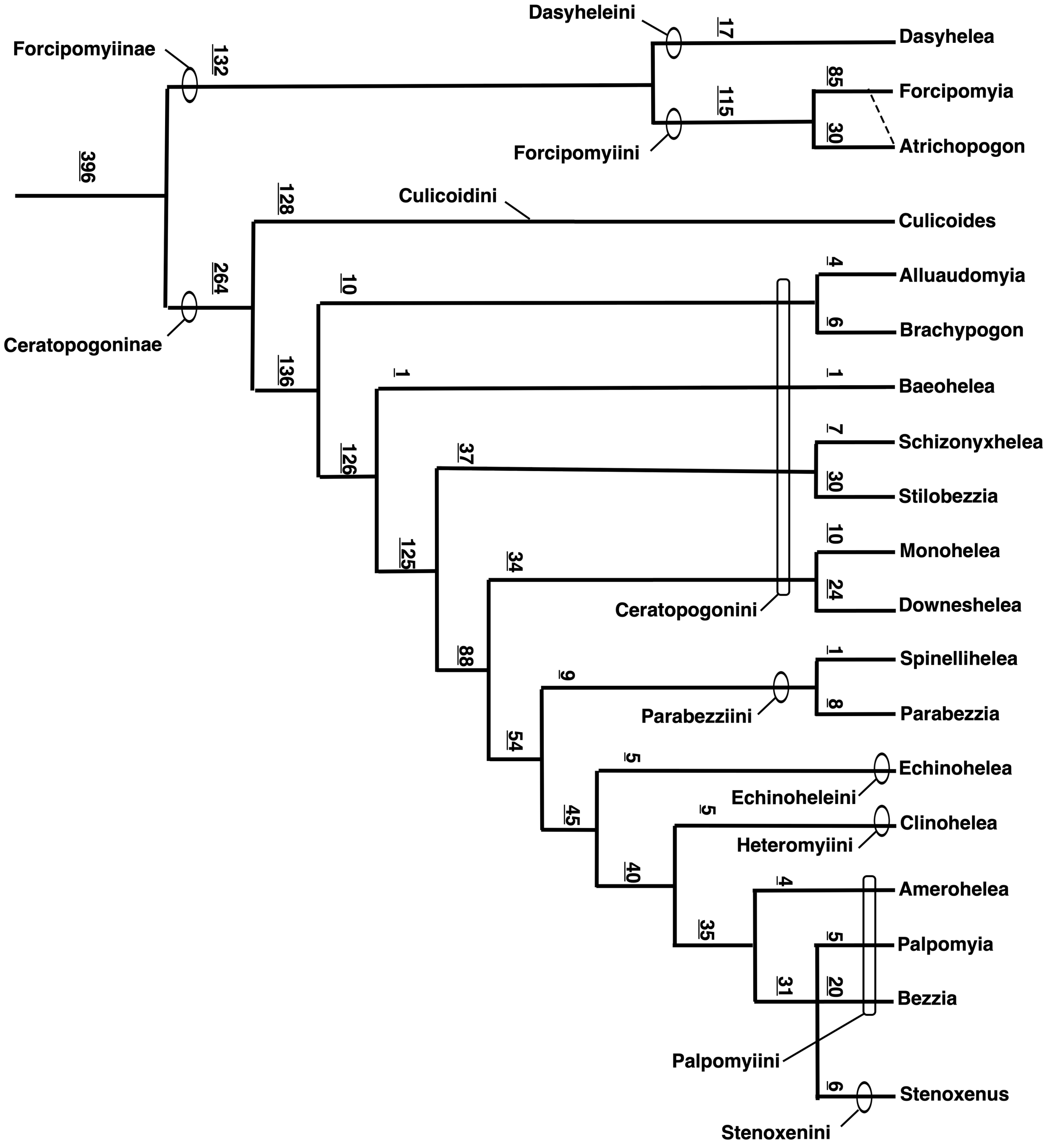


FIGURE 7. Phylogeny of the genera at Zurquí and Tapantí, Costa Rica indicating numbers of named species known in Costa Rica at each bifurcation.

The Ceratopogonidae have had a recent cladistic analysis allowing for a better understanding of the diversification of the family at the generic level (Borkent 2024). Lineages present at Zurquí and Tapantí (Fig. 6) show that of the 245 species present, most belong to the Forcipomyiinae (n= 150) and within this subfamily, most are Forcipomyiini (n= 119) with *Forcipomyia* and *Atrichopogon* being nearly equally diverse. It is important to note, here and in the following discussion that there is a distinct possibility that *Forcipomyia* is paraphyletic in relation to *Atrichopogon* (Borkent 2024). The sister group of the Forcipomyiinae includes 95 species at Zurquí and Tapantí, placed in the Ceratopogoninae. Although within this subfamily the earliest lineage is the genus *Culicoides* and with 28 species is the most diverse at the genus level, in reality its sister group is the more diverse lineage with 67 species. Further bifurcations within the Ceratopogonini and subsequent tribes indicates a pattern of each bifurcation being composed of a significantly more diverse lineage and its sister having far fewer species. The terminal bifurcation is between the Heteromyiini with two species and the Palpomyiini + Stenoxenini with 29.

How do the cloud forest Ceratopogonidae compare to those in the broader Costa Rican fauna? Figure 7 provides a phylogeny with the numbers of named species in all of Costa Rica in the genera present at Zurquí and Tapantí (the lowland genera not present at Zurquí and Tapantí are discussed below). Of the 396 species in these genera (Table 3), the Forcipomyiinae with 132 species are notable less diverse compared to their sister lineage of Ceratopogoninae with 264. Within Forcipomyiinae, the Forcipomyiini are similarly more diverse than *Dasyhelea* but within the tribe, *Forcipomyia* is nearly three times as species-rich as is *Atrichopogon*, certainly a result of lack of taxonomic study of the latter. Within Ceratopogoninae, *Culicoides* with 128 species is nearly as diverse as its sister lineage of 136 species, again differing from the pattern in Zurquí and Tapantí. Subsequent bifurcations within the remaining Ceratopogoninae indicates a similar pattern of sister group relationships between a much more diverse lineage and one of limited diversity. The influence of the diversity in *Stilobezzia* and the results of recent revisions of *Downeshelea* describing many new species (Santarém *et al.* 2018, 2020) are evident at two bifurcations.

There are 16 genera of Ceratopogonidae in Costa Rica which were not collected at Zurquí and Tapantí and which are found at lower elevations (Table 3). However, these add only 34 species to the total of 430 named species known from this country. Other than *Paryphoconus* Enderlein, the sister group of *Stenoxenus*, with six species, the other 15 genera have 1–4 species each. They generally do not add significantly to the pattern found in figure 7. However, it is notable that there are seven species of Johannsenomyiini, the sister group of Palpomyiini + Stenoxenini (Fig. 8, Table 3), present in the low and middle elevations of Costa Rica. Similarly, the rest of the fauna of the Neotropical Region is composed of relatively small genera which do not substantially change the pattern noted here (Table 3). Of the 18 genera apparently absent in Costa Rica, 11 are restricted to Patagonia and/or the southern, temperate region of South America (*Paradasyhelea* Macfie, *Austrohelea* Wirth & Grogan, *Borkenthelea* Spinelli & Grogan, *Isthmohelea* Ingram & Macfie, *Macrurohelea* Ingram & Macfie, *Notiohelea* Grogan & Wirth, *Diaphanobezzia* Ingram & Macfie, *Yungahelea* Spinelli & Ronderos, *Physohelea* Grogan & Wirth, *Austrosphaeromias* Spinelli, *Sphaerohelea* Spinelli & Felippe-Bauer). *Ceratoculicoides* Wirth & Ratanaworabhan (Huerta & Borkent 2005, Fasbender 2023) and *Nannohelea* Grogan & Wirth (pers. obs.) are known but mostly undescribed from Costa Rica. *Lanehelea* Wirth & Blanton is known only from Colombia.

Worldwide, the early lineage of Forcipomyiinae has 2,295 named species (Fig. 8). The sister group of Forcipomyiinae has 3,824 species and Culicoidini (including *Culicoides* with 1,360 species and *Paradasyhelea* with only 11), forming the next lineage in the phylogeny has 1,371 named species, with its sister group of remaining Ceratopogoninae including 2,454 species. Similar to the pattern noted above, subsequent bifurcations generally form sister groups with one lineage having far more species than the other (Fig. 8). The paraphyletic Ceratopogonini with 1,205 species is composed of numbers of polytomies and bifurcations and there is a possibility of a more balanced number between sister groups once such genera as *Alluaudomyia*, *Brachypogon*, *Stilobezzia* and the *Monohelea* complex are more fully described. Beyond the Ceratopogonini, the bulk of the 1,248 species within the remaining tribes are in Johannsenomyiini + Palpomyiini + Stenoxenini.

Examination of photographs of the ACG BINs showed that of 1060 BINs present, 977 were identifiable to genus and an additional 40 to Ceratopogoninae other than *Culicoides*. Of these, 136 were *Dasyhelea*, 527 *Forcipomyia*, 175 *Atrichopogon*, 77 *Culicoides* and 102 were in genera in the remaining Ceratopogoninae (62 identified to genus, 40 to Ceratopogoninae other than *Culicoides*). Keeping in mind that 43 BINs could not be identified based on low resolution photographs, these provide proportions that are significantly different than is known for the cloud forest species at Zurquí and Tapantí and for named species elsewhere, with these four genera composing at least 86% of the BINs. If the number of BINs is an accurate portrayal of species, it would likely indicate that many more species in these four genera can be expected proportionally elsewhere. However, further sampling, especially with trapping methods other than Malaise traps is needed to test this further.

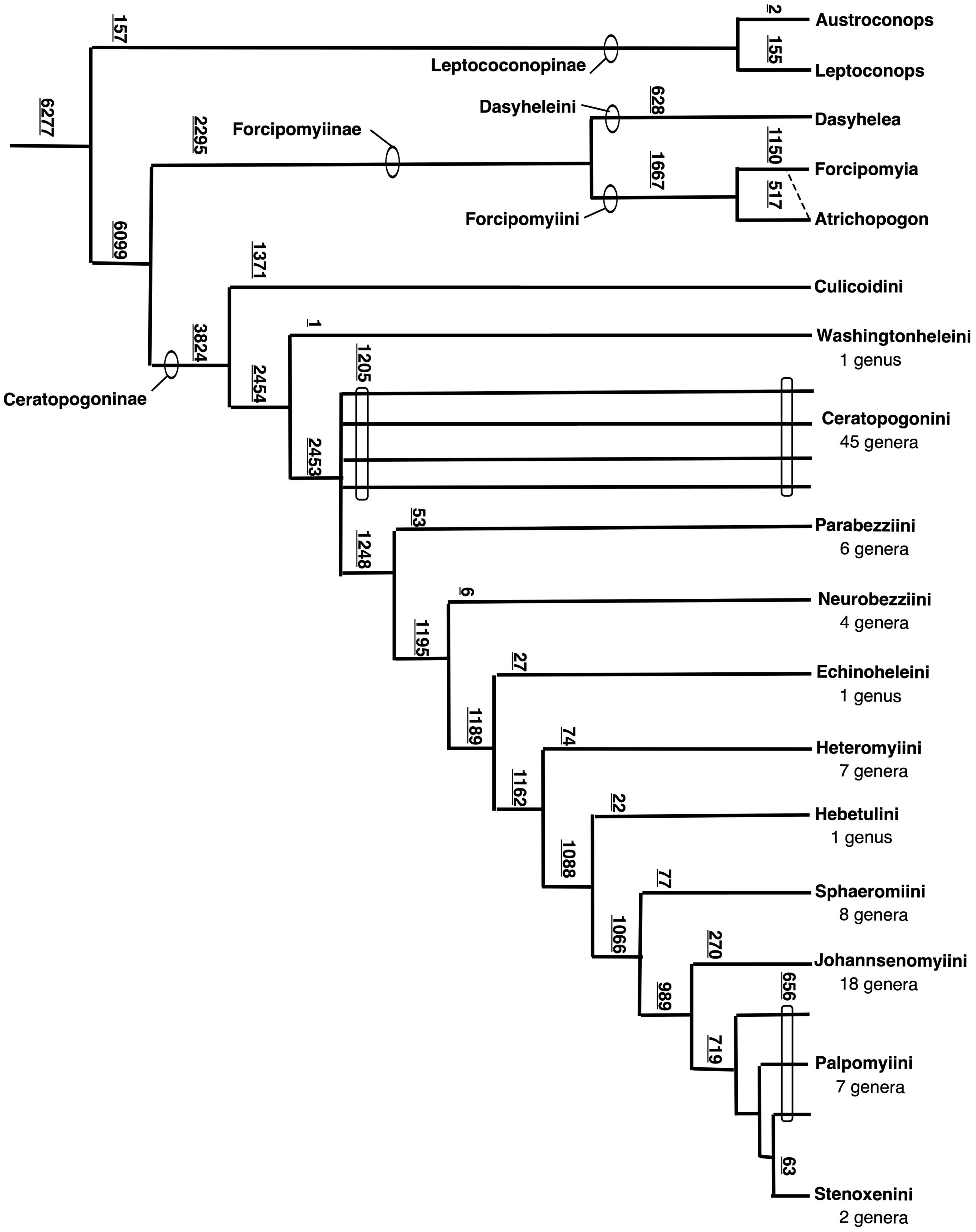


FIGURE 8. Phylogeny of the genera and tribes of Ceratopogonidae of the world, indicating numbers of named species at each bifurcation. Numbers are based on Borkent & Dominiak (2020) and Borkent *et al.* (2022).

Dasyhelea, *Atrichopogon*, *Forcipomyia* and *Culicoides* formed 72% of the named and unnamed fauna of Zurquí and Tapantí and 61% of the named Costa Rican fauna. This follows a general pattern within the family in which, worldwide, these four genera, representing early lineages within the family, include 58% of all named species. Why would these genera be comparative more diverse in the cloud forests at Zurquí and Tapantí compared to named species in all of Costa Rica and worldwide? One corresponding feature reflects the habitat of the immatures. The larvae of these four genera are generally in small aquatic and semiaquatic habitats such as phytotelmata, rock pools, mud at the margin of pools, marshes, seeps, springs and steams, in wet moss, rotting vegetation and, for some *Forcipomyia*, in moist terrestrial habitats such as under bark of dead wood and in other and various moist to wet substrates. Members of the Ceratopogonini are also often in smaller aquatic habitats but increasingly in higher tribes, the preponderance of taxa are in larger lotic and lentic habitats, including in the benthos of small and moderately-sized lakes (Borkent 2014). It is likely because cloud forest habitats generally do not have lakes of any size, nor generally bigger streams or rivers, members of Heteromyiini, Sphaeromiini, Johannsenomyiini and Palpomyiini and Stenoxenini are generally absent from high elevations or are represented by only a few species that do occur in smaller habitats such as phytotelmata. Further to this, species that live in smaller, temporary habitats tend to have greater dispersal levels. As such, Borkent (1991) showed that it is *Dasyhelea*, *Atrichopogon*, *Forcipomyia* and *Culicoides* which occur on oceanic islands of volcanic origin; islands of continental origin have a much greater number of genera present. These associations with small temporary habitats have likely contributed to the diversification of these four genera and, in the case of the cloud forests of Zurquí and Tapantí, have allowed these lineages to invade habitats not generally available to many low and middle elevation groups. It is striking that among the higher lineages present at Zurquí and Tapantí, the Palpomyiini are quite diverse, with 27 species, nearly equal to the number of species of *Culicoides*. There are a number of members in this tribe that have, as interpreted here, reverted to small aquatic habitats (e.g. *Bezzia* and *Palpomyia*; Campos *et al*. 2011, Lane 1946, Spinelli & Wirth 1991, Ronderos *et al*. 2004). It was striking that all 16 species of *Bezzia* at Zurquí and Tapantí were in the *B. gibbera* species group (= *Bezzia bivittata* species group). Some other species in this group have been reared from a swamp (*B. gibbera*) or from a bog (*B. luteiventris*) (Borkent 2014, Spinelli & Wirth 1990, Wirth & Grogan 1983). A number of others have been swept as adults from the margins of small streams (Wirth & Grogan 1983). These habitats are reminiscent of habitats present at Zurquí and Tapantí.

Finally, lower numbers of some of the higher tribes of Ceratopogoninae in Central America, where lakes are scarce in areas both north and south of this region, perhaps provides zoogeographic barriers to dispersal. This too may limit the potential lineages present in the cloud forest of Costa Rica.

How Do Different Trapping Methods Compare in Sampling Species of Biting Midges?

Although Malaise traps are often the collecting method of choice for many studying Diptera, this very productive trap yet fails to sample some taxa. Although it is true that many studying species of *Culicoides* use light traps, often combined with CO2, this method clearly fails to sample many other taxa that either do not feed on vertebrates, including many of those species of *Culicoides* that do not feed as females, or are otherwise restricted. In addition, virtually none of those studying *Culicoides* are interested in the other genera of Ceratopogonidae and fail to report the results of light trapping for these other taxa.

There have been very few studies comparing collecting methods of Ceratopogonidae. However, one outstanding example was by Knausenberger (1987) who, in a remarkably intense collecting program centered in Virginia, USA, reported 193 species of Ceratopogonidae in Virginia, West Virginia, North Carolina and Alabama, including 43 unnamed species. This represents 30% of the 648 known Nearctic species (Borkent & Dominiak 2020, Borkent *et al.* 2022). He reared 1,100 individual larvae and pupae and another 7,059 unassociated immatures to adults, sampled with a wide array of methods from many different habitats. He also collected 5,950 other larvae and pupae and sampled 2,765 adults with seven different types of traps including UV and incandescent light traps, suction trap, interception trap, sweep net, aspiration of biting females and emergence traps. Although 82 of the 193 species were reared, the methods he employed did not lend themselves to comparative numbers and the relative efficacy of the trapping methods. Rather, his approach was to understand the total fauna in as much depth as possible, attempting to understand the autecology of each species.

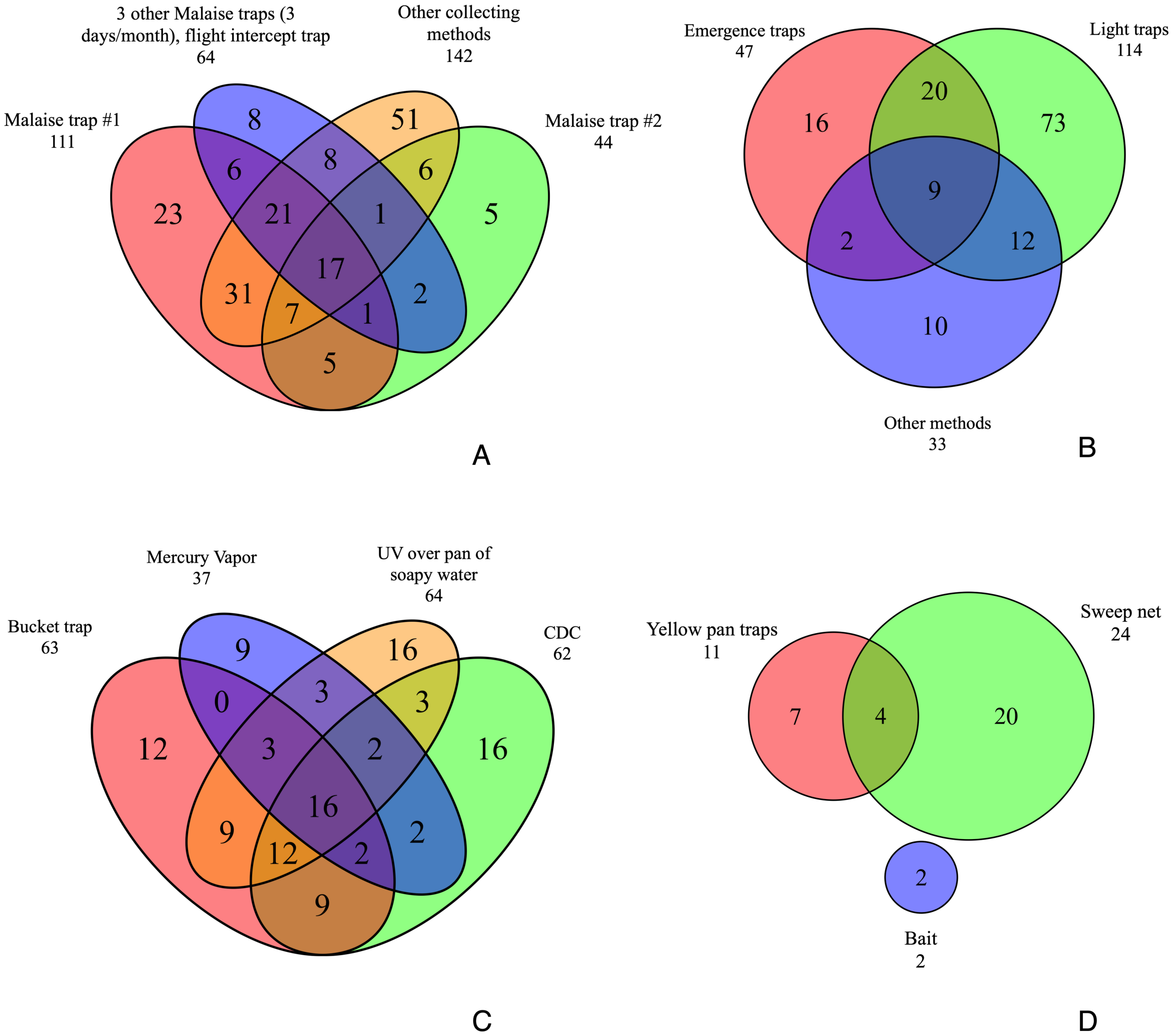


FIGURE 9. Numbers of species collected with different trapping methods at Zurquí, Costa Rica. A. Comparison of Malaise trap #1, #2, combined other Malaise traps, and other methods. B. Comparison of emergence traps, light traps and other methods other than Malaise traps. C. Comparison of different light traps (UV= ultraviolet; CDC = Center for Disease Control). D. Comparison of yellow pan traps, sweep net and bait.

Kitching *et al.* (2004) showed that Malaise traps collected significantly more species of Ceratopogonidae than pyrethrum canopy knock down and far outperformed yellow pan traps in Queensland, Australia and in Papua New Guinea. Elbers & Meiswinkel (2014) compared female *Culicoides* sampled from a cow and a sheep with that of a UV light trap in the Netherlands and showed that the numbers of specimens per species ranked similarly but with significantly different periodicity and host preference. Viennet *et al.* (2011) found the same, studying sheep in France and Scheffer *et al.* (2012) also found that light trapping under-represented some *Culicoides* species compared to aspiration from horses in South Africa. Tilki & Dik (2003) studied the effect of different colours of light traps sampling female *Culicoides* in Turkey and found significant differences in relative attraction between some of them. Venter *et al.* (2018) found differences in species of *Culicoides* present and their abundance when comparing variously coloured LED light traps and the "Onderstepoort" light trap in South Africa and cite a number of other papers studying *Culicoides* in this regard.

The 2,830 specimens slide mounted and studied from Zurquí came from a variety of traps during the year of sampling (Figs. 9A–D) and allows for comparison of the differing methods, similar to what was done for the entire

Diptera fauna by Borkent *et al.* (2018). Of the 192 species at Zurquí, 111 were sampled with Malaise #1 (at the forest margin) and 44 from Malaise trap #2 (in the ravine) with 30 of the latter also sampled by Malaise trap #1 (Fig. 1A). Other Malaise traps (those sampling only 3 days a month) sampled 64 species with 8 of these being unique and an additional 9 sampled only by other collecting methods. Considering that Malaise traps collected 141 of the 192 species (73.4%) (Fig. 9A), this collecting method is clearly the collecting method of choice for large numbers of species. However, 51 of 192 species (26.6%) were sampled only by other methods, with 32 of these sampled only with light traps, seven only from emergence traps, four only from sweeping, two only from baits (*Dasyhelea* ZUR-3 and *Forcipomyia cornuta* Saunders from honey sprayed on vegetation and fruit bait, respectively) and one from a yellow pan trap (far more effective in some other families of Diptera but here collecting the only representative of *Parabezzia*). Four were collected with both light traps and emergence traps and one by both sweeping and a light trap. These 51 species not sampled by Malaise traps included 4 *Dasyhelea*, 15 *Atrichopogon*, 11 *Forcipomyia*, 9 *Culicoides*, 1 *Alluaudomyia*, 1 *Brachypogon*, 1 *Downeshelea*, 1 *Monohelea*, 3 *Stilobezzia*, 1 *Parabezzia*, 1 *Spinellihelea* Borkent, Grogan & Picado, 1 *Bezzia* and 2 *Palpomyia*. It is notable that these unique taxa include the only *Alluaudomyia*, 1 of 4 *Brachypogon*, 1 of 4 *Monohelea*, 3 of 6 *Stilobezzia*, the only *Parabezzia*, the only *Spinellihelea*, and 2 of 6 *Palpomyia* recorded from Zurquí. If this is a general pattern for the family, the striking report by Srivathsan *et al.* (2023) that their study of Malaise trap samples of Ceratopogonidae showing that they are the second most diverse of all insect families worldwide significantly increases their estimate for this family.

A total of 114 species were collected with eight light traps sampled for three days per month (Fig. 9B), compared to the year-round Malaise trap #1 sampling of 111 species, showing the efficacy of this collecting method. The most intense light, that of the Mercury vapour light, sampling 37 species (Fig. 9C) often seems too bright for some Ceratopogonidae and doesn't appear to be as effective as the dimmer lights from the CDC, bucket and UV lights each of which sampled somewhat more than 60 species. Mercury vapour lights at sheets often attracts Ceratopogonidae that land at some distance (often more than a meter) from the light itself (AB pers. obs.). Nevertheless, in our study two species of *Forcipomyia* and two species of *Culicoides* were sampled only with mercury vapour lights.

Emergence traps caught 47 species (Fig. 9B) and this method provides more detailed information about the breeding habitats of a given species. In addition, it ensures that the species sampled actually are present in the habitat and weren't sampled as adults blown into the area. Emergence traps also may sample species not collected by other methods (here seven species). There are surprisingly few studies of Ceratopogonidae using emergence traps, especially considering how popular they are with chironomid workers. Havelka (1976) placed emergence traps over small streams at two localities 115 kms southwest and 59 kms east of Frankfurt, Germany and sampled 62 and 55 species respectively, for a combined total of 70 species. Havelka & Caspers (1981) found 53 species from two years of trapping from a small woodland stream at a third locality, 130 kms northwest of Frankfurt, Germany and an additional 20 species not sampled by Havelka (1976). In total, these emergence traps collected 90 species (including three previously unnamed species) associated with small streams, represent 35% of the total ceratopogonid fauna recorded from Germany. Knausenberger (1987: 4) cites work by Masteller & Wagner (1984) and personal communication regarding emergence traps employed for one year to sample 50 species from two small effluent streams near Lake Erie, Pennsylvania, USA. These studies suggest that using this technique is a productive means of interpreting the diversity of Ceratopogonidae.

Although using a sweep net resulted in only 26 species (and only four unique) (Fig. 9D), this method was used rather infrequently at the very wet Zurquí site and belies how effective it is in reality, as experienced by the first author who uses this method predominately (see also sampling at Carrasco National Park in Bolivia, discussed below, Table 5). Sweeping also has a major benefit of often providing important clues as to the breeding habitat of collected species (especially when sampling males which often are near the site of emergence).

Table 8 presents the number of species of each genus collected by the various methods, reflected in more general terms in figures 9A–D. There were some notable differences between the genera. For example, Malaise traps were particularly effective for *Dasyhelea* and moderately so for *Forcipomyia*, while Malaise traps and light traps collected similar numbers of species of *Atrichopogon* and light traps somewhat more than Malaise traps for *Culicoides*. Light traps were effective in sampling species of *Stilobezzia*, *Bezzia* and *Palpomyia*. However, it is important to emphasize that these comparisons of different trapping methods are not objective comparisons, considering the numbers of traps and the duration of their use differed significantly (see Materials and Methods).

TABLE 8. Number of species captured by different sampling methods at Zurquí, Costa Rica. Genera are in tribes arranged in phyletic sequence. Methods: MT, Malaise and flight intercept traps; LT, light trap; EM, emergence traps; SN, sweep net; YP, yellow pan trap; BT, bait trap.

Genus	**Methods**					
	MT	**LT**	**EM**	**SN**	**YP**	**BT**
Dasyhelea	18	4	5		2	1
Atrichopogon	28	25	8	6	2	
Forcipomyia	44	29	12	14	2	1
Culicoides	20	26	5	4	1	
Alluaudomyia		1	1			
Baeohelea	3	1	2		1	
Brachypogon	3	1	1			
Downeshelea	1	1	1			
Monohelea	2	3	2			
Schizonyxhelea	1	1				
Stilobezzia	3	6	2		1	
Parabezzia					1	
Spinellihelea		1				
Echinohelea	1	1				
Clinohelea	2					
Bezzia	9	8	3		1	
Palpomyia	6	6	5			
Totals	141	114	47	24	11	2
Proportion of 192 spp.	73%	59%	24%	13%	6%	1%
# unique spp.	50	32	7	4	1	2

Comparison of Diversity During a Year at Zurquí

There are no phenological data available for tropical Ceratopogonidae as a group and virtually nothing on their life cycles (Borkent 2017, Borkent & Spinelli 2007). The temporal abundance of adults of species of *Culicoides*, however, have been studied in a few other tropical countries (e.g. Forattini *et al*. 1958—Brazil, Diarra *et al*. 2015—Senegal, Dipeolu 1976—Nigeria, Chatterjee *et al*. 2018—India (also interpreting *Dasyhelea*), Harsha *et al*. 2020—India) and these all at lower elevations. There are none at high altitude that we are familiar with.

The ZADBI project extracted only certain groups in their entirety but a number of families, because of their abundance, were selected for curation and further study based on whether they possibly represented a further species. The Ceratopogonidae were one of these latter families. As such, our abundance data is highly biased and is not based on total numbers collected. Nevertheless, we consider it likely that the numbers of specimens extracted are yet a crude expression of abundance and we present it here for Zurquí Malaise trap #1 (Fig. 10A) and Tapantí (Fig. 10B), both of which were permanent and on the forest edge.

These two graphs provide a very limited perspective of what may possibly be a phenological pattern in Costa Rican cloud forests. However, we cannot tie this to any meteorological data as this was not collected. The rainfall patterns in Costa Rica are complex and vary significantly on the Pacific and Atlantic coasts, becoming locally complex (and variable) in highlands (Savage 2002).

The BOLD data from the ACG study provide a more consistent basis for examining the phenology of Ceratopogonidae as a group because all specimens were extracted from the Malaise traps. The markedly dry tropical forest of lowland Guanacaste province is represented by the samples from Bosque San Emilio (at 300 m) (Fig. 11A) where the influence of the wet season from May–November (Steyn *et al*. 2016) is represented by smaller numbers in June and July of both 2012 and 2020 but is very evident in the high adult emergence in October–December in 2020.

The Malaise trap at San Gerardo (575 m, rain forest) (Fig. 11B) exhibited highest adult numbers in September–March 2013/2014 but this was not repeated in 2014/2015. The trap at Derrumbe (1500 m, cloudforest) and the most similar to those from the ZADBI project (Figs. 10A, B), appears to have more consistent emergence of adults over the year, although with higher numbers in March–June in both 2015 and 2016. Figures 13A, B and 14A, B present the sequential collection of adults at the geothermal platform at PL12 (791–853 m, old growth, mixed dry and rain forest) from November 2013–November 2020. An ad hoc visual examination of the graphs suggests that adult emergence may be concentrated in May–July (evident in 2016–2020 and somewhat in 2015) and occasionally in November–December (evident in 2014, 2016 (also into January), 2017, 2018, 2019).

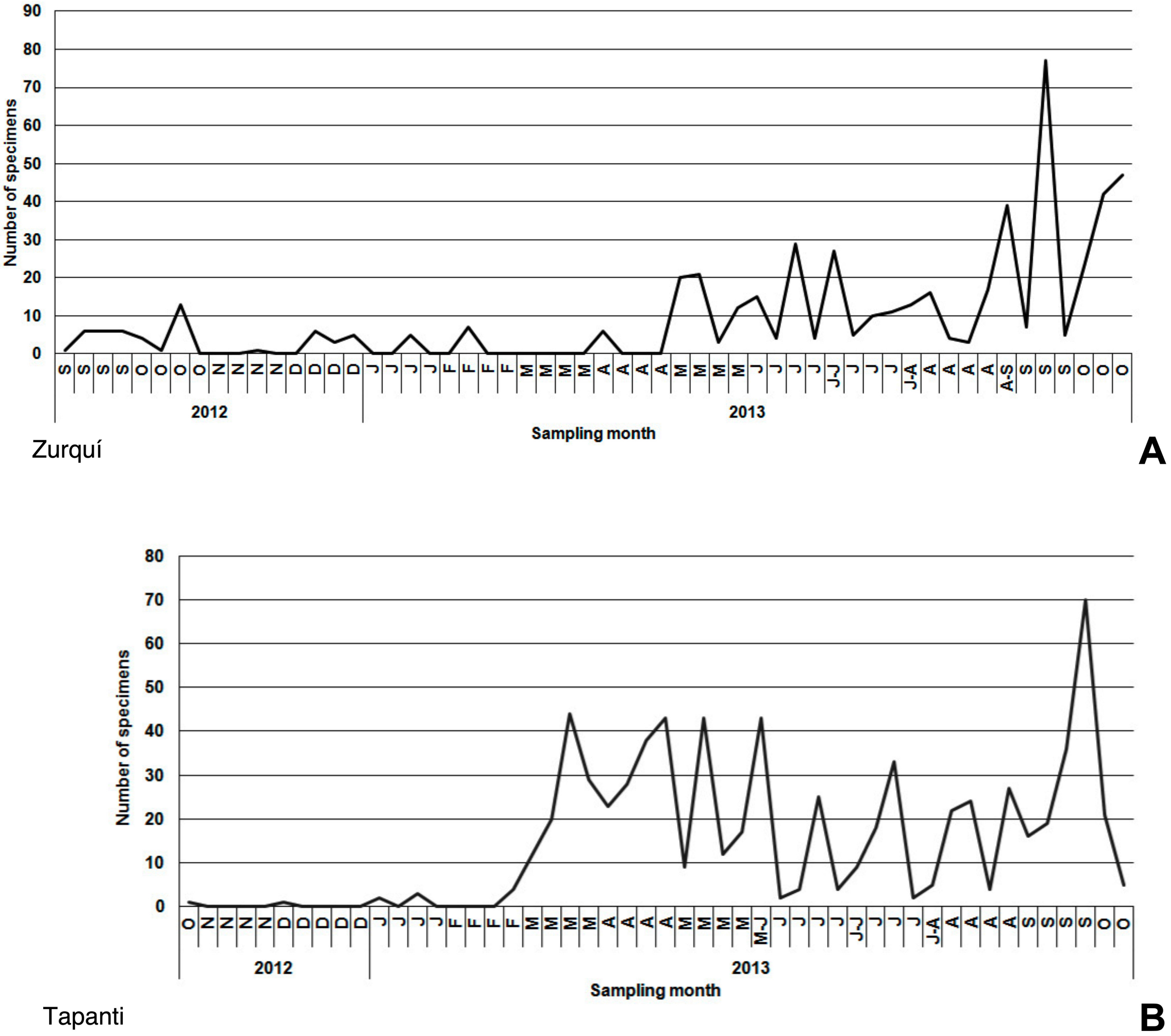
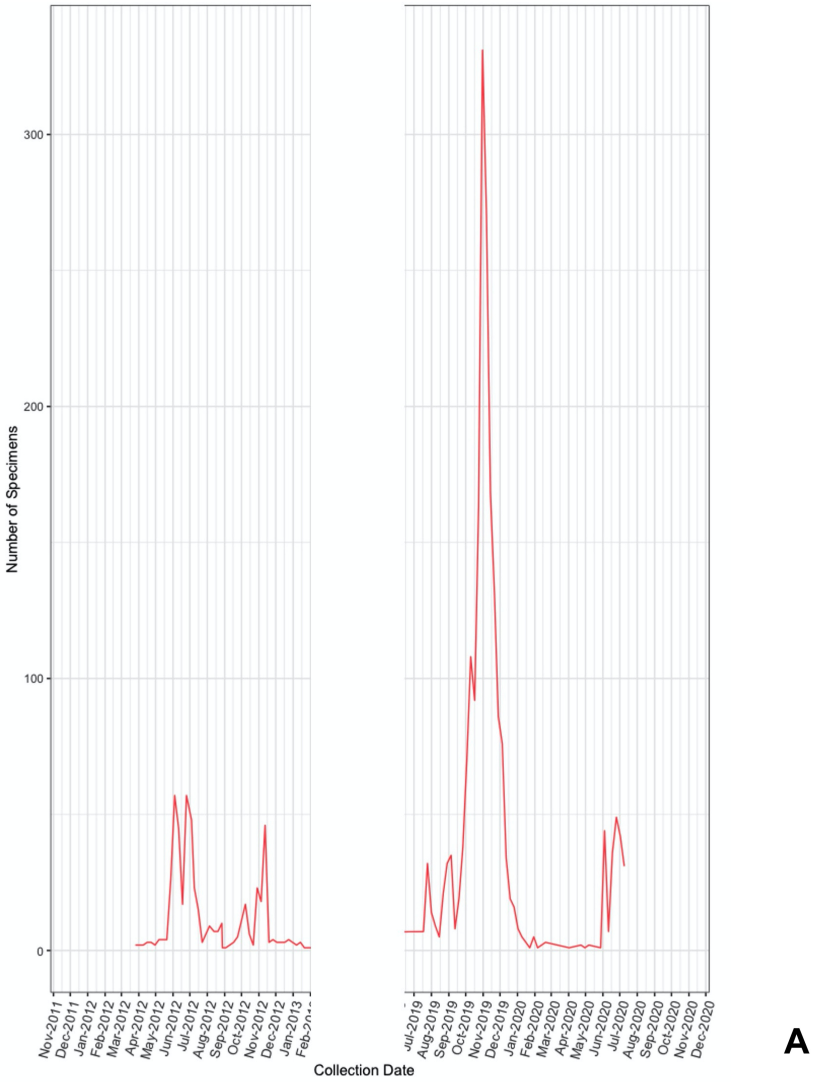
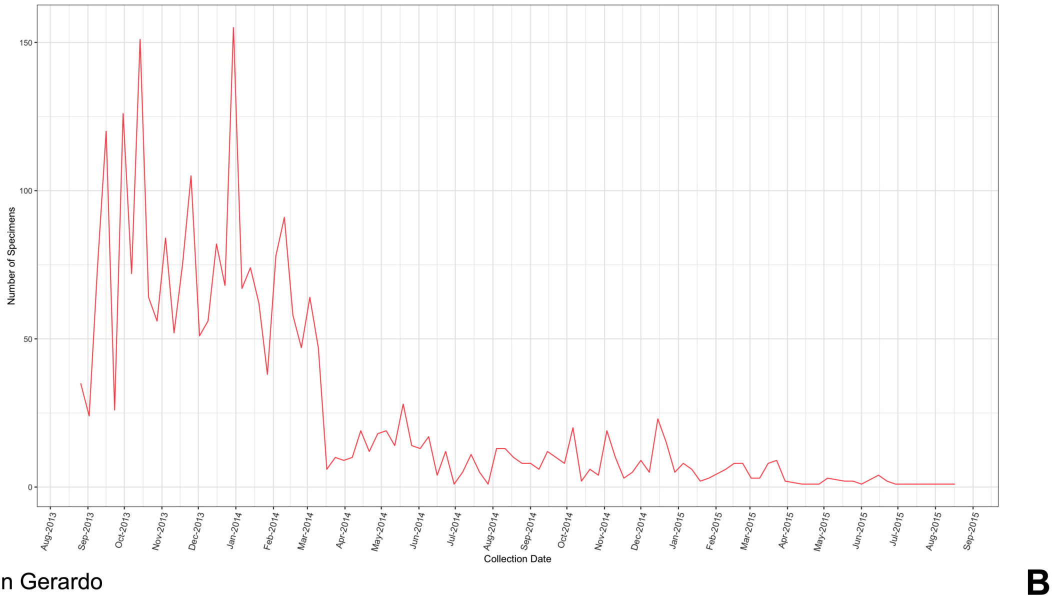


FIGURE 10. Temporal distribution of specimens selected from samples at A. Zurquí, Costa Rica. B. Tapantí, Costa Rica. X-axis with first letter of month indicated.

It is important to emphasize that examining the phenology of an entire family of insects (or any group of species) as a unit is not the most logical basis for understanding their emergence patterns. In areas with extremes of climate, such as in temperate regions and as seen here for the seasonally dry forest present in the lowlands of Guanacaste province in Costa Rica (Fig. 11A), suddenly favourable conditions will coordinate the emergence of the adults of many species. Each species, of course, has its own life cycle and therein lies an open field of inquiry, especially for Ceratopogonidae occurring in more humid tropical conditions.



Bosque San Emilio



San Gerardo

FIGURE 11. Temporal distribution of specimens from the Área de Conservación Guanacaste, Costa Rica. A. Bosque San Emilio, 300 m, secondary successional Pacific dry forest. B. San Gerardo, 575 m, rain forest. Further details are provided in Materials and Methods.

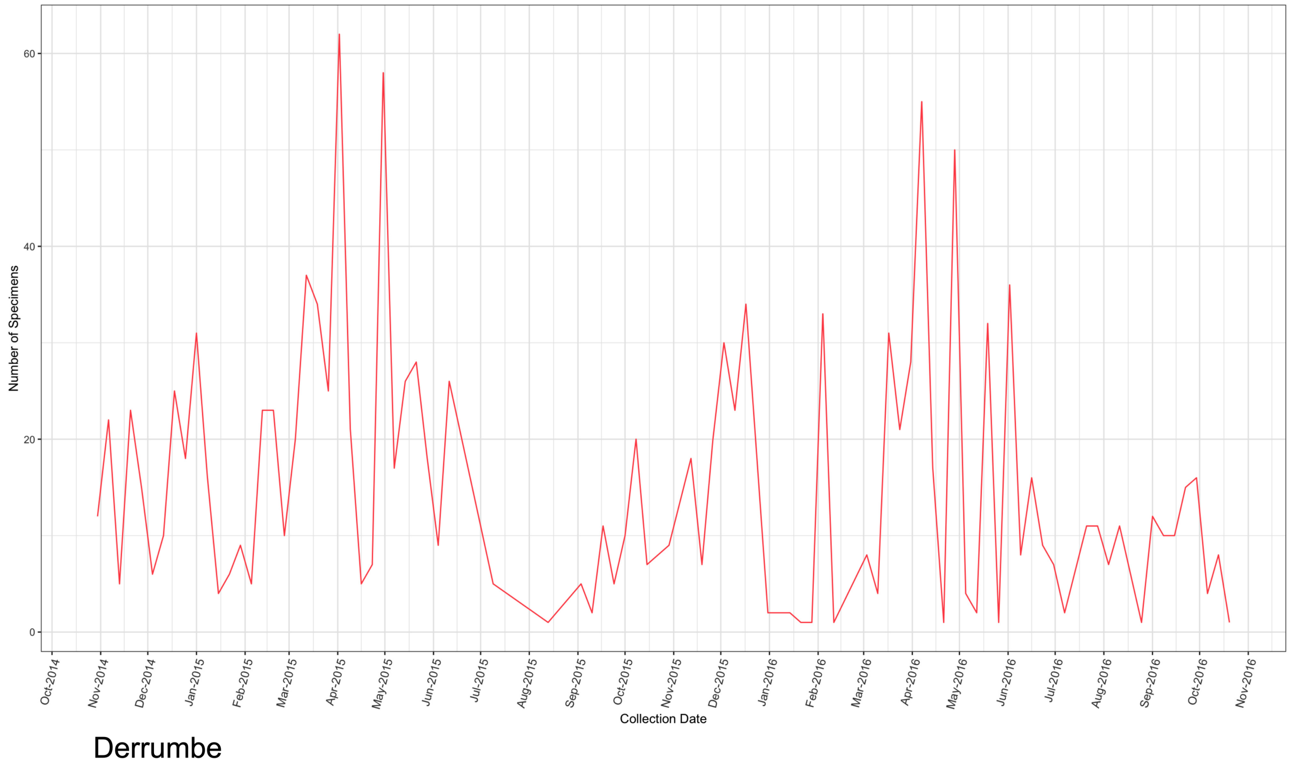


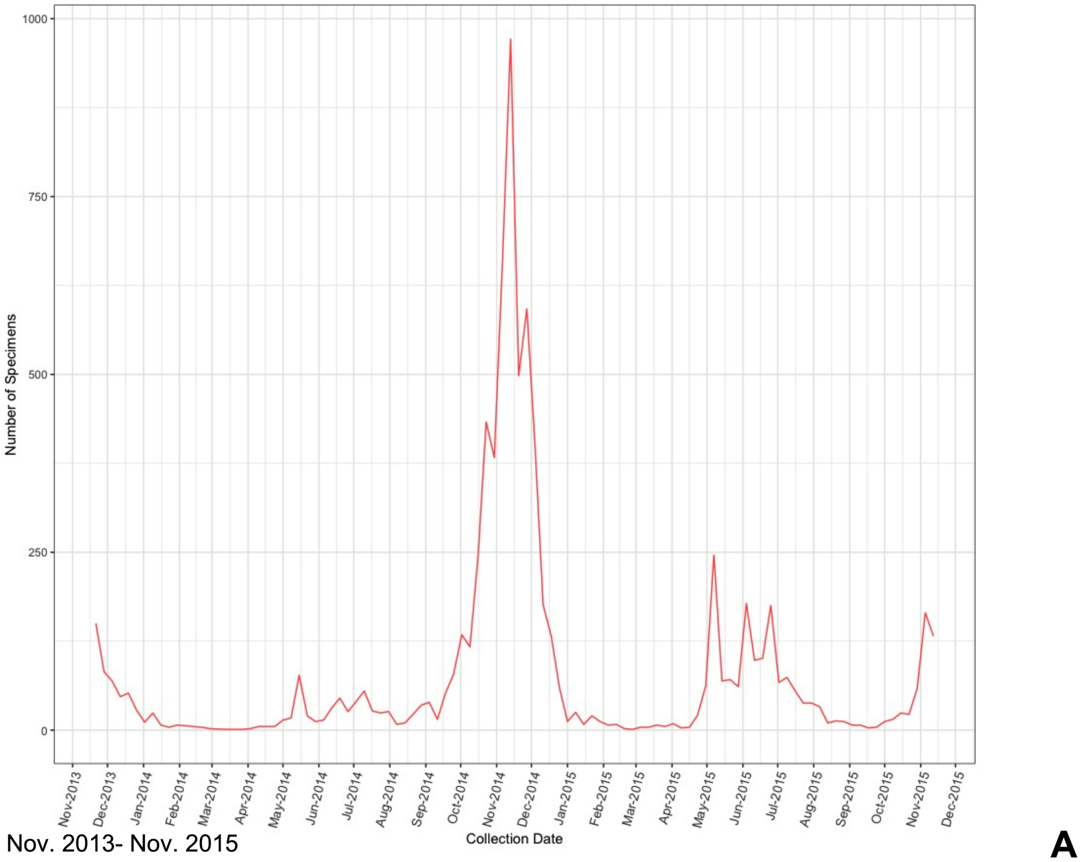
FIGURE 12. Temporal distribution of specimens from Derrumbe at 1220 m, cloud forest in the Área de Conservación Guanacaste, Costa Rica. Further details are provided in Materials and Methods.

Of the very few detailed phenological studies available for Ceratopogonidae as a family, in Germany, Havelka (1976) and Havelka & Caspers (1981) noted how different two years may appear, likely depending on temperatures.

Relative Abundance of Species at Zurquí

As just noted above under "Comparison of diversity during a year at Zurquí", the selection of specimens to maximize the number of morphospecies strongly biases comparisons of relative abundance. Nevertheless, it was clear that some species were rather common but that the strong majority were rather rare. Figure 15 depicts the numbers of species sampled at Zurquí (all traps) and their relative frequency. Of the 192 species and 2415 specimens at Zurquí, 58 (30%) were recognized on the basis of a single specimen each. A total of 116 (60%) were sampled by four or fewer specimens, 145 (76%) by nine or fewer and 162 (84%) by 15 or fewer specimens. One prominent species, *Culicoides covagarciai* was represented by 576 specimens and was abundant in many samples from each of the Malaise traps, each of the light traps, was collected with a sweep net and yellow pan trap and was present in emergence traps over dry and wet branches, leaf litter, other vegetation and stagnant water, indicating that the immatures are likely in wet soil. In spite of this broad ecological niche and its distribution from Honduras to Colombia and Venezuela, the immatures of this species have not been described, reflecting the large gap in our understanding of the immatures of Ceratopogonidae (Borkent 2014). Of the 47 species with 10 or more specimens each, nearly all were sampled with both at least one Malaise trap and one light trap. The only exceptions were two species of *Dasyhelea*, one *Forcipomyia*, two *Culicoides*, one *Downeshelea* and one *Bezzia* which were collected just with a light trap or with both light and emergence traps.

The 58 singletons collected strongly suggests that there are significantly more species at Zurquí than sampled here. Unfortunately, because the specimens were selected, it is not appropriate to do a rarefaction analysis to estimate how many species might be expected, as was done for those families of Diptera in the ZADBI project which were completely extracted from the samples (Brown *et al.* 2018).



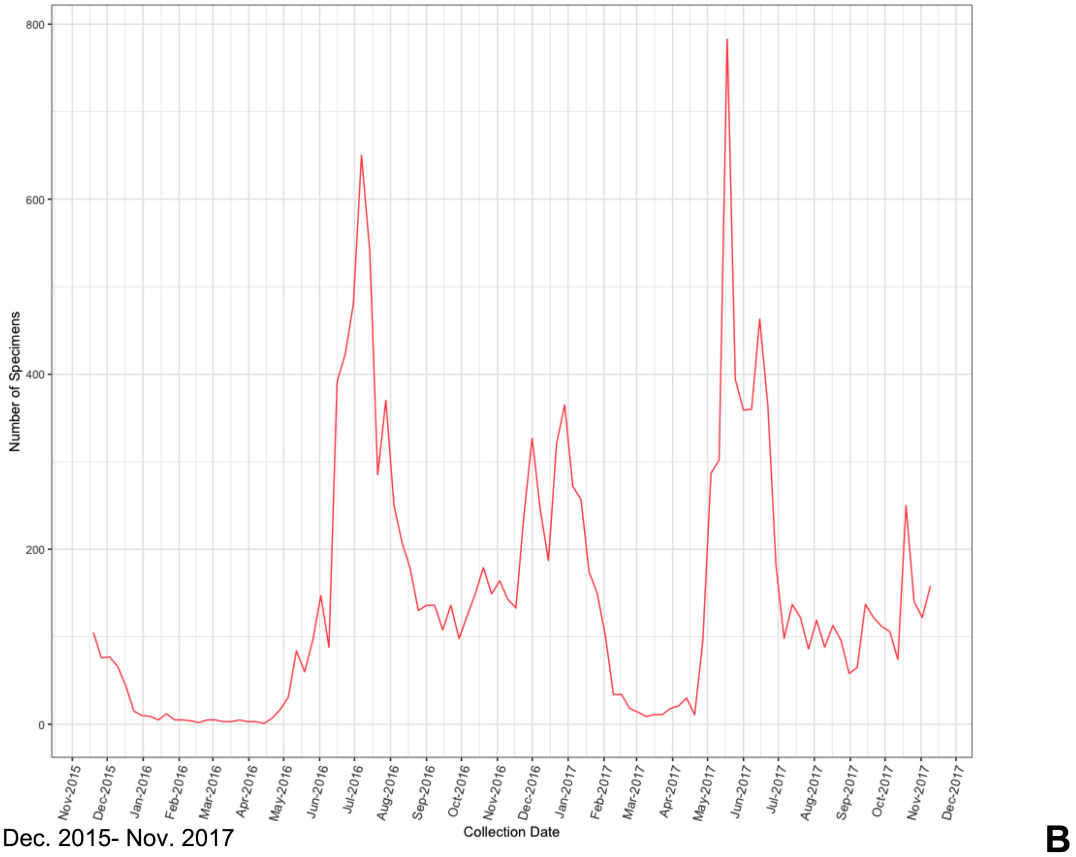
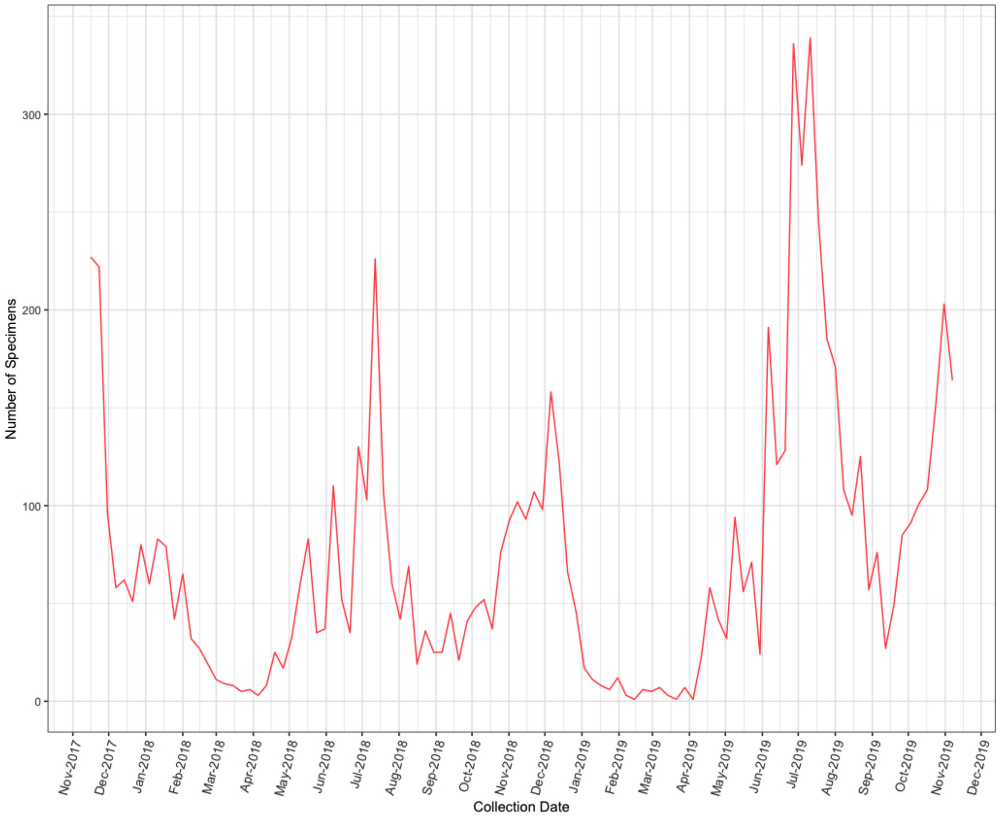
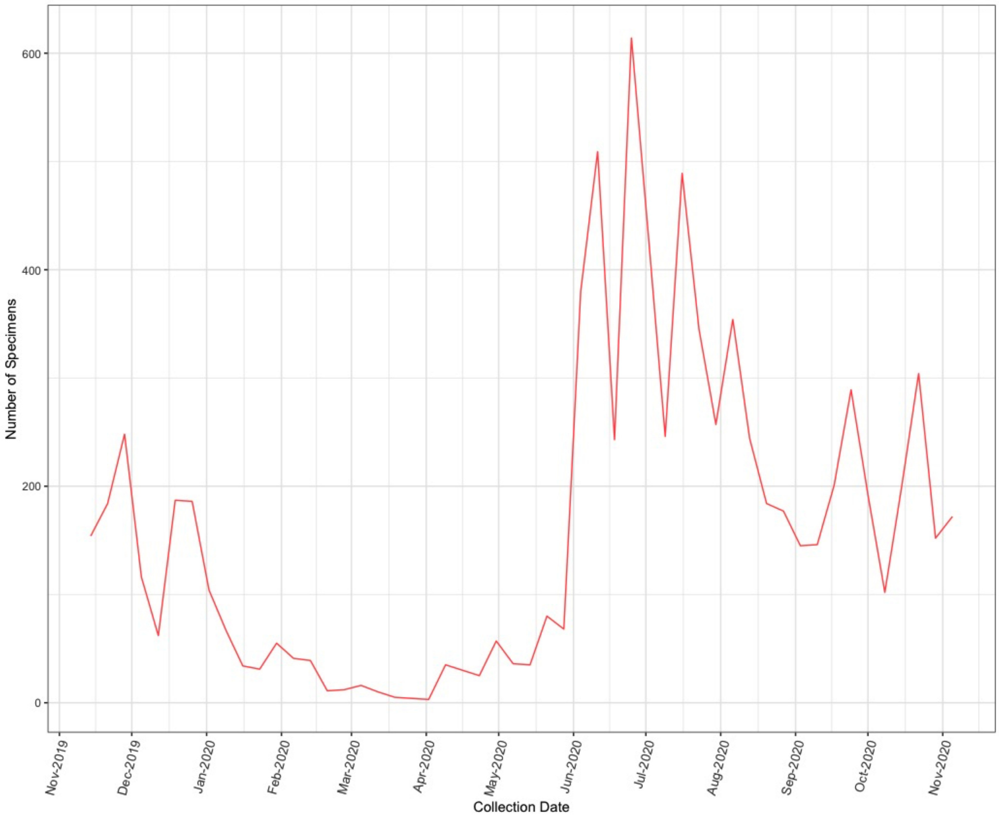


FIGURE 13. Temporal distribution of specimens from around the geothermal development platform (PL12) in the Área de Conservación Guanacaste, Costa Rica. A. November, 2013–November, 2015. B. December, 2015–November, 2017. Further details are provided in Materials and Methods.



Nov. 2017- Nov. 2019

A



Nov. 2019- Nov. 2020

B

FIGURE 14. Temporal distribution of specimens from around the geothermal development platform (PL12) in the Área de Conservación Guanacaste, Costa Rica. A. November, 2017–November, 2019. B. November, 2019–November, 2020. Further details are provided in Materials and Methods.

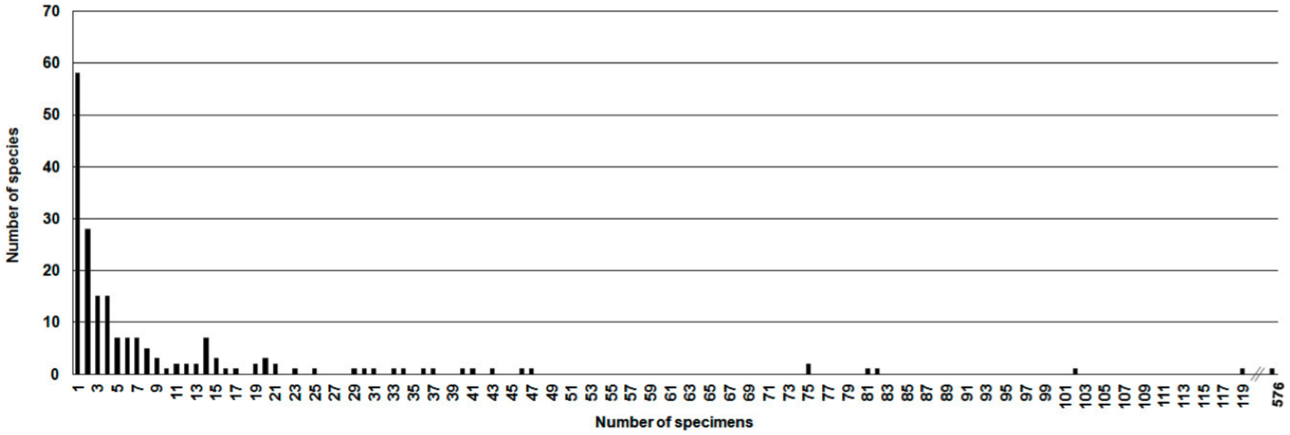


FIGURE 15. Number of specimens sampled of named and unnamed species at Zurquí, Costa Rica.

Conclusions

Considering that most species of Ceratopogonidae live in the tropics, it is surprising how little we know about them. With the exception of some pest species of *Culicoides*, we know virtually nothing about their life cycles, their habitat restrictions, elevational distribution and a host of other aspects. Fewer than 15% of named Neotropical species are known as either pupae or larvae, meaning for the remainder we have no idea where their primary habitat is. This is strikingly incomplete when compared to other biting fly families where the immatures of most (Simuliidae; about 90% each for Neotropical larvae and pupae; Adler pers. comm.) to nearly all (Culicidae) are known and carefully described.

This study of the Ceratopogonidae of the cloud forests from the ZADBI project and DNA barcodes of species from the ACG in Costa Rica opens a few windows as to the nature of its tropical fauna. The 192 species of Ceratopogonidae at the cloud forest of Zurquí represents the highest number of species of this family from any location in the world and the combined 245 species from Zurquí and Tapantí are remarkable compared to the 430 named species for all of Costa Rica and 1,314 named species from the entire Neotropical Region. The report of 1,060 BINs from Malaise traps at four locations in the ACG and 4,023 BINs from the ACG combined with a limited number of sites from elsewhere in Costa Rica (Figs. 2A, B) (Janzen & Hallwachs 2019) further indicates huge diversity within Costa Rica. It appears to be reasonable to suggest that there are conservatively at least 5,000 species in this country. Further, striking numbers of species in single or small areas in Bolivia, on the eastern slopes of the Andes, also indicates remarkably high numbers of species, yet to be discovered and named. Only two other families from the ZADBI project have been interpreted in any detail. Epler (2017) recognized 137 species of Chironomidae with 98 (72 %) undescribed, in 63 genera (17 new). Grimaldi & Richenbacher (2023) discussed the Drosophilidae, reporting 352 species from Zurquí, compared to 305 named species for all of Costa Rica, so that it is clear that this family too is in great need of further systematic study.

So based on these numbers, how many species of Ceratopogonidae could be estimated worldwide? In better known groups, with it being more likely that total numbers are closer to being complete, the Costa Rican fauna and flora makes up about 5% (Culicidae; Wilkerson *et al.* 2021), 1% (Simuliidae; P. Adler pers. comm.), 5% (Odonata; Paulson *et al*. 2024), 4% (Anura; Savage 2002) and 3% (vascular plants; Qian *et al.* 2022) of the world's named species. Comparison of the named Costa Rican Ceratopogonidae (n= 430) with named worldwide species (n= 6,267) gives a percentage of 6.9% and if this is applied to the 5,000 species from Costa Rica estimated here, it suggests that there are actually nearly 73,000 species worldwide. However, if the ratio is around 5%, as it is for Culicidae, a group restricted to lentic habitats, and Odonata, with species in broader aquatic environments, it would give an estimate of 100,000 species worldwide. If Ceratopogonidae are compared to Simuliidae, which are at 1% and restricted to flowing water, it would indicate the presence of 500,000 species of Ceratopogonidae, but this is highly improbable and likely reflects the significant differences in the ecology of these two families.

There are about 62 named Costa Rican Chironomidae (Epler pers. comm.; Watson & Heyn 1993, Epler 2017) and these represent about 1% of the world fauna. In many ways the Chironomidae are more similar to Ceratopogonidae in the diversity of habitats occupied by their immatures (although Chironomidae are still significantly broader). However, the 62 species are undoubtedly an extreme underestimate of the true number in Costa Rica. Watson & Heyn (1993) named only 21 species but indicated the presence of 148 species, presumably many of them unnamed. Epler (2017) provided a detailed report of the chironomids from the ZADBI project and found 137 cloud forest species, 98 of them (72 %) undescribed, in 63 genera (17 of which were considered new). Clearly, the group needs significantly more systematic study before it could be used to extrapolate numbers for the Ceratopogonidae.

These high numbers would be in line with the results obtained by Srivathsan *et al.* (2023) based on barcoding samples from Malaise traps showing that Ceratopogonidae are the second most diverse of all insect families worldwide, after Cecidomyiidae. Other recent studies also indicate an astonishing diversity of Cecidomyiidae, with Hebert *et al.* (2016) concluding that this family may have 1.8 million species worldwide (based on barcoded specimens in Canada and extrapolating based on this country having 1% of species globally) and cecidomyiids by far the most species rich at Zurquí, with 800 species but with this number capped by the overwhelming number discovered and therefore incomplete (Borkent *et al.* 2018, Brown *et al.* 2018). Further conjecture is possible but what is needed are complete surveys and good systematic studies to provide more objective data, throughout tropical countries and particular in those known to be rich otherwise (e.g. the eastern slopes of the Andes, East Africa and much of the Oriental Region). Regardless, the estimate by Borkent (2017) of at least 9,000 undescribed species appears to be a decidedly inadequate underestimate of the true number of species of Ceratopogonidae living on our planet.

There is a serious question as to the value of pursuing and evaluating the number of species of Ceratopogonidae and other groups. It is certainly impressive to realize the extent of diversification and that there are many species yet to be discovered. It is an incentive to preserve habitat and suggests that much of our biota is under continuing threat of extinction. Furthermore, it is a warning to conservation biologists and others working in forestry and agriculture not to simplify their models to the strikingly few generally considered. Nature is far, far more diverse and complex than we previously thought.

However, it is important to remember that a catalog of names or a list of barcodes is a very limited understanding of life on our planet. A poignant question remains: what is the biological significance of determining the number of species yet to be named other than indicating the extent of the task at hand? And what is the nature of that task? As biologists, our job is to understand nature as it is and each of these species has a story to tell, about its morphology, phylogenetic relationships, adaptations, behaviour, ecology and much more. Even having representatives of each preserved in museums, as some are anxious to do before they disappear, is of limited value, similar to interpreting fossils. Once extinct we'll never really know how these species lived and functioned. The loss of this life, or being content merely with knowing only numbers of unnamed species, results in a serious deficit in our understanding of evolutionary models and all the biological features which might be interpreted in knowing their phylogenetic relationships. If half of the approximately 6,400 species of mammals were unknown or known only by a number, what would we deduce regarding their zoogeographic and bionomic divergence and so much more? In short, what is desperately needed now is an army of systematists who can describe the plethora of currently unnamed species and place them in an evolutionary context, allowing for further and undoubtedly more complex models of understanding of the biodiversity around us. The model proposed by Caruso *et al*. (2024) in which barcoding and morphological studies are complementary is clearly the way of the future and especially so for certain genera of Ceratopogonidae, such as *Atrichopogon* which are hugely diverse but generally appear to be morphologically monotonous as adults (not so as immatures).

There are further considerations regarding the community of Ceratopogonidae present in the cloud forests at Zurquí and Tapantí. It is important to remember that the concept of cloud forests as a community, like other broad community definitions, is a human perception. The vast majority of species in these communities are ecologically independent of one another, responding to environmental conditions, including interspecies interactions, individually. With the exception of, for example, some parasites and tight pollination relationships, each species responds independently according to its needs as the environment changes, modifying its distribution in different ways.

Even so, some habitats as characterized by humans are often somewhat predictive. As such, the ZADBI project compared cloud forest at 1,600 meters from three localities in Costa Rica with the assumption that these high elevation communities would share stronger similarities than with lowland communities and thereby give a more accurate portrayal of species turnover.

However, many species are not so compliant and there are often significant questions regarding what makes up a community of species. In the northern temperate region, plant ecologists have designated an array of increasingly refined forest types (e.g. spruce/hemlock or cedar/hemlock) that do not include many other species that may or may not be present. In broader categories, such as the Arctic Biome, many species are restricted to this habitat but even so, many are not. Studies of Quaternary subfossil insect communities have shown combinations of species in communities that are entirely unknown today (Elias 1994). This is true for larger organisms as well. For example, musk-ox bones have been found with oak and butternut tree remains south of the Wisconsin ice sheet 12,000 years ago, (Semken *et al.* 1964), where today they are separated by 1,500 kms. Interglacial species of beetles in Great Britain are now present some distance away, including species restricted to a Nepal–Vietnam distribution (Coope 2010). It seems clear that this would also be true for species of Ceratopogonidae, with each responding to changes individually and reconfiguring various combinations and associations over time and space. As such, our comparisons between Zurquí and Tapantí, based on two cloud forest communities at 1600 m, are likely somewhat artificial. We can reasonably expect that communities in the tropics would present similar and likely even greater challenges of interpretation as they reconfigure and redistribute through time.

Here we report that the Ceratopogonidae of Zurquí includes some species that are endemic, some shared with just the cloud forest at Tapantí but that others are found much more broadly, including habitats at lower elevations. It emphasizes the point that the ecology and distribution of species must be understood individually to obtain a more accurate picture of biodiversity.

Further to this it is uncertain how many of the species, sampled only as adults, are actually living at Zurquí and Tapantí. The ratio of males to females provides clues as to the area sampled by a given trap. Borkent (2000) discussed the evidence that males form about 40% of emerging adult Ceratopogonidae both now and as represented by a variety of ambers. Because males remain largely in the area from which they emerge, while females disperse (especially those of early lineages in temporary habitats), the ratio of males steadily decreases with distance from the site of emergence. Only about 26% of the BOLD specimens from the ACG samples discussed here were males, suggesting that these Malaise traps sampled dispersing females emerging from elsewhere—what distance is unknown but potentially many kilometers. However, these data are potentially biased because they are based on a single photo representing each of the 1,060 BINs; it is possible that there are associated males that we did not see photos of for at least some of the females. Because specimens were selected, we have no data on the percentage of males and females collected during the ZADBI project.

The depiction of both temporal distribution of the Ceratopogonidae at Zurquí and Tapantí and the number of specimens/species sampled from Zurquí were based on strongly biased data because specimens were preferentially selected based on whether they might represent another species. As such, there is a great need to explore the life cycles and abundance of Ceratopogonidae in all tropical regions.

One of the great threats regarding extinction of species is that so many are now in “islands” of habitat, often defined by humans as forming certain communities, but with nowhere to go as climate and conditions change. One thing is certain, communities do not change their distributions as a group of species; habitat connections and corridors, proposed to connect such islands of habitat, likely function far better for vertebrates than for the vast majority of insects.

Suggestions for Future Research

The most obvious research suggestion that leaps out of our publication is the need for basic systematic studies: namely to collect, identify, interpret phylogenetically and then interpret zoogeographic and bionomic divergence and further interpret the remarkable diversity of Ceratopogonidae which this study indicates remains largely undescribed. This portrayal of Ceratopogonidae informs other researchers of various smaller taxonomic units that could be revised. Revisions are needed for every genus and the ZADBI material, beautifully mounted on slides, will be an important addition.

Some museums hold large numbers of undescribed species, most notably the Smithsonian, that are waiting to be studied. The Canadian National Collection and that in the Museo Nacional de Costa Rica are also rich in uninterpreted material. What marvels might yet be discovered can only be a provocation to further study. The group, diverse in species numbers and ecological expression, is clearly an excellent candidate for evolutionary and ecological studies.

The strong majority of named species, let alone those undescribed, are unknown as immatures and their significant role in various habitats where the larvae feed and where the species spends most of its time during its life cycle, are therefore completely unknown (even though the adults may be present in large numbers).

Readers will recognize the difference in the approach of the ZADBI project which interpreted the morphology of the species and providing identifications based on their known taxonomy, compared to that of the DNA barcoding, which generated only generic level identifications. The ceratopogonid taxonomists on this paper (AB, GRS, FD) examined the photos associated with the BIN numbers for material from the Área de Conservación Guanacaste and provided these generic names which were then utilized to make some broader observations here. There is no hope of determining the actual species without these specimens being slide mounted and examined by a skilled ceratopogonid systematist and for which, at the present time, there is no support (logistical, financial). With the increasing decline of such expertise, it is difficult to see how such identifications will ever be provided before the specimens deteriorate in the ethanol in which they are currently preserved and can no longer be made into good microscopic slide preparations. Further to this, because only 26% of the 1,060 the Área de Conservación Guanacaste specimens, for example, were males, many will be uninterpretable taxonomically to the species level using morphology. Considering that males often provide markedly superior (and in some groups, the only) means of identification of a species, this provides another barrier to incorporating this information into a broader and comprehensive systematic framework. It is unfortunate that they are not fully curated at the present time, so an investment in that regard now would be of great benefit in later years.

A point made above is that we are uncertain which species collected as adults with various traps are living in the immediate area, nor what their primary habitats as immatures are. Barcoding immatures and tying these to adults would provide a wealth of information regarding the true nature of the biodiversity of Ceratopogonidae in a given area and potentially specific information on the specializations of many species.

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