HOW MANY SPECIES OF *DROSOPHILA* (DIPTERA, DROSOPHILIDAE) REMAIN TO BE DESCRIBED IN THE FORESTS OF SÃO PAULO, BRAZIL? SPECIES LISTS OF THREE FOREST REMNANTS.

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Abstract

This paper describes the composition and abundance of *Drosophila* species found in three forest remnants in the State of São Paulo. A well-standardized sampling procedure applied on four collecting trips to the same sites on four areas resulted on 944 samples. All males collected were identified by analyses of the genitalia, this being the only data set used. One hundred and twenty five species were detected amongst the 29,289 males analyzed. From them 57,6% could be identified as described species. Thirteen of the species found were absent from the previous species list for the state of São Paulo State, thus represent an increase of 13% on the number known. We argue that the majority of the 53 unidentified species are in fact undescribed. The sites studied did not differ significantly in the proportion of identified species. On average identified species were almost seven times more abundant than unidentified ones, and this difference was significant. Rarefaction curve analysis confirmed that the proportion of unidentified species increase with sample size, and did not reach a plateau with our data set. These results illustrate the large richness of *Drosophila* species in forest remnants of São Paulo State. It also indicates that about half of the species in this region remain to be described. This conclusion is particularly important when one considers that this is a well studied genus of Diptera, on the best sampled region of Brazil.

Key words: *composition; diversity; richness; inventory; atlantic forest; neotropical region.*

Resumo

Este trabalho descreve a composição e a abundância de espécies de *Drosophila* encontradas em três remanescentes florestais do estado de São Paulo. Um procedimento de coletas bem padronizado aplicado em quatro coletas nos mesmos sítios em três áreas resultou em 944 amostras. Todos os machos coletados foram identificados pela análise da genitália, e apenas os dados destes foram analisados. Cento e vinte e cinco espécies foram detectadas entre os 29.289 machos analisados. Destas 57,6% puderam ser identificadas como espécies já descritas. Treze das espécies encontradas estavam ausentes da lista prévia de espécies do estado de São Paulo, resultado em um aumento de 13% nesta lista. A maioria das 53 espécies não identificadas são, provavelmente, não descritas. Os sítios estudados não diferem significativamente na proporção de espécies identificadas. Em média as espécies identificadas foram quase sete vezes mais abundantes do que as não identificadas, e esta diferença foi significativa. Uma análise de curvas de rarefação confirmou que a proporção de espécies não identificadas aumenta com o tamanho amostral, e não atinge um platô em nosso conjunto de dados. Estes resultados ilustram a grande riqueza de espécies de *Drosophila* nos remanescentes florestais do estado de São Paulo. Eles também indicam que cerca de metade das espécies desta região ainda não foram descritas. Esta conclusão mostra-se particularmente importante considerando que este é um gênero de Diptera bem estudado, na região mais bem amostrada do Brasil.

Palavras-chave:*: composição; diversidade; riqueza; inventário; mata atlântica, região neotropical.*

Introduction

With more than 1700 species (Tidon-Sklorz & Sene 1999), the *Drosophila* genus has been historically explored by geneticists. Nowadays there is increasing interest on applying these well studied and easily manipulated animals on the study of the distribution of biological diversity and its causes (e.g. Sevenster & Alphen 1993; Shorrocks & Sevenster 1995; Worthen *et al.* 1998). This new application for *Drosophila* makes the publication of faunistic inventories of this taxon particularly relevant.

A series of local inventories of *Drosophila* faunas has been provided for South America (Dobzhansky & Pavan 1943; Pavan & Cunha 1947; Malogolowkin 1951; Pavan 1959; Vilela *et al.* 1980; Val & Kaneshiro 1988; Tidon-Sklorz *et al.* 1994; De Toni & Hofmann 1995; Tidon-Sklorz & Sene 1995; Val & Marques 1996; Goñi *et al.* 1998; Vilela & Mori 1999). Data on the distribution of species has also been provided by papers dealing primarily with other questions. Some reviews about the theme have also been published (Sene *et al.* 1980; Val *et al.* 1981; Vilela *et al.* 1983; Tidon-Sklorz & Sene 1999; Vilela *et al.*.002).

The *Drosophila* fauna of the neotropical region is highly diversified (Val *et al.* 1981), with numerous species remaining to be described. Many of these species can be distinguished only by analysis of the male genitalia (Vilela 1992). Male genitalia, especially the aedeagus, is the most important character used by taxonomists to recognize, describe and synonymize species (see Vilela & Bächli 1990). Indeed differentiation on male genitalia can be presented as a gross description for the species concept most frequently applied in this taxon. This also occurs with other animal taxa and results from the common and widespread pattern of faster and divergent morphological evolution of these structures (Eberhard 1985).

The practice of using this character in *Drosophila* taxonomy is also based on other evidences. Geneticists have carried out tests of reproductive isolation in the lab, and on the field with the application of genetic markers. In many cases, when sibling species are detected on basis of genetic markers or reproductive isolation, differentiation of the male genitalia is observed (eg. Spassky 1957). Thus the male genitalia are the tool of choice for identifying species in neotropical *Drosophila* communities.

In this paper we provide species lists for three sites, which represent the three major types of forest found in the state of São Paulo, southeastern Brazil. These lists are the first inventories in Brazil we have notice, with a defined sampling design and standardized collecting method, in which ***all*** collected individuals were identified by analyzing the male genitalia. Our results show an increase of 13% in the number of species compared to the previous species list for this State.

Material and Methods

Collecting Methods - *Drosophila* were caught using a trap developed to minimize bias in capturing different species of flies attracted to banana baits (Medeiros & Klaczko 1999). This trap showed better results compared to others tested, especially for capturing species that resist entering traps, such as those of the *tripunctata* group. Traps were baited with peeled, ripe bananas fermented with dried baker yeast (*Saccharomyces cerevisiae)* for 36 h (50 g of yeast per 5 kg of banana). Each trap received 100-150 ml of bait and was hung at 10 cm from the forest floor for 24 h, after which the animals caught were removed. The baits, and the parts of the trap that had been in contact with it, were then changed for a new collecting turn. On each collecting trip, traps were set on three consecutive days to provide a total of 72 h of sampling. The traps were hung close to the forest floor to mimic naturally decaying fruits. The bait was replaced daily with fresh fermented banana, to allow the comparison of the results from consecutive days without the confounding effect of bait aging.

The sampling design was aimed primarily at studying the association between the taxocenosis and a gradient of humidity. Thirty points were sampled at each site, equally distributed between 10 classes of distance from a stream: 1; 2.5; 5; 10; 20; 40; 60; 80; 120; and 160 m. These collecting points were not distributed in lines, but on a design that allows a minimal distance of 40 m between adjacent traps. The resulting sampling area was a rectangle of 9.6 hectares (600 m long, bordering the stream, and 160 m wide, perpendicular to the stream). No traps were set at less than 3 m from glades.

Study areas - Samples were obtained from three sites at different forest remnants in the state of São Paulo, southeastern Brazil. These localities which differ clearly in their climatic and geomorphological conditions, as well as on their respective vegetation, represent the three major types of forest formations in these state (Salis *et al*. 1995), namely, the interior plateau forests, represented by Barreiro Rico farm; forests on the western slopes of the Serra do Mar mountains and those of the the Serra da Mantiqueira mountains, represented by Serra do Japi at over 1000 m (Leitão-Filho 1992; Rodrigues & Shepherd 1992); and forests of the eastern slopes of the Serra do Mar mountains, represented by Ilha Bela.

Throughout this region the climate can be divided in two main seasons: a cold and dry from April to September, and a hot and humid from October to March (Cezar & Leitão Filho 1990; Morellato 1992).

Barreiro Rico farm (B. Rico in the rest of this paper) includes three fragments of well preserved semideciduous forest (total of 2,200 ha). The sample fragment was about 336 ha. The topography is plane, with an average altitude of about 500 m. The average annual precipitation is 1,339 mm,

and the average temperature 21.5°C. The maximum average mothly temperature occurs in February (31.3° C) and the minimum in July (11.7°C) (Cezar & Leitão Filho 1990).

The Serra do Japi (Japi in the rest of this paper) includes a group of mountains belonging to the Mantiqueira complex. The Serra do Japi park has an area of 19,170 ha. The altitude within it ranges from 700 to 1300 m, and the climate (Pinto 1992), soil (Rodrigues & Shepherd 1992) and vegetation (Leitão-Filho 1992; Rodrigues & Shepherd 1992) vary markedly with the altitude. The average annual temperature at higher sites is around 15.7° C, with the maximum average monthly temperature occurring in January (between 18.4° C and 22.2° C), and the minimum in July (between 11.8° C and 15.3° C). The rainfall varies considerably over short distances, with an overall estimate of about 1500 mm per year.

The Parque Estadual de Ilha Bela (I. Bela in the rest of this paper) is located on an island off the northern coast of São Paulo State (23°47' S and 45°24'W). The topography is mountainous, with 73% of the island between 100 and 900 m above the sea-level (maximum of 1379 m) (França 1951). The park covers an area of 27,025 ha which corresponds to about 80% of the island. The maximum monthly precipitation occurs in December (2000 mm), and the minimum in August (79 mm). The average monthly temperature varies from 19.5°C in the winter to 24°C in the summer (Tommasi 1985 apud Belúcio 1995). The vegetation is included in the region of the "Floresta Ombrófila Densa" (IBGE 1983).

The sampling area in B. Rico was on the southern margin of a section of a stream located between 48°05'11"W - 22°41'15"S and 48°04'52"W - 22°41'26"S. In Japi and I. Bela, the sampling areas were on the northern margins of streams. The coordinates of the sections of the streams used were: Japi - between 46°57'48"W - 23°14'06"S and 46°57'33"W - 23°14'12"S; I. Bela - between 45°20'49"W - 23°50'48"S and 45°20'30"W - 23°50'47"S.

Four collecting trips were made to each site — two in the hot/humid season and two in the dry/cold season. At B. Rico, the sampling periods were January 6-9, March 2-5, June 13-16, July 23-26, in 1998. At Japi they were February 21-24, March 18-21, July 7-10, and August 12-15, in 1998. At I. Bela, the periods were December 19-22 1997, January 15-18, June 22-25, and September 2-5, in 1998.

Identification of Samples - Each collected male was identified by analyzing the genitalia, the only reliable method for recognizing many neotropical *Drosophila* as pointed out by Vilela (1992) in the case of the *tripunctata* group. The fresh aedeagus (the intromittent organ, and the most useful part of male genitalia for species identification) of at least one individual of each species found was observed under a microscope and drawn with the help of a camera lucida. This drawing facilitated the identification of the species, which was based on figures of male genitalia available in the taxonomic literature. Species for which no corresponding aedeagus could be found in the literature received a code, and the other individuals found from these species were identified by comparing them with our drawings and/or with the first collected specimens. Nevertheless, in most cases the genitalia were analyzed under a stereomicroscope with no need for removal, as proposed by Spassky (1957). In two cases, the identification was not based on the shape of aedeagus: the distinction between *D. melanogaster* Meigen 1830 and *D. simulans* Sturtevant 1919 was based on the shape of the posterior salience of the genital arch (Salles 1948), the shape of the hypandrium was used to distinguish between the species of the *willistoni* complex (Burla *et al.* 1949; Malogolowkin 1952; Spassky 1957). Since the identification of most species was not based on original descriptions, we have indicated the published figures of male genitalia used for the identification (see table 1).

The flies were analyzed fresh on a film of water in a petri dish. Since the external characters were not analyzed, and since no preparation of genitalia parts was necessary, considerable velocity compatible with identification of big samples was possible. Specimens are preserved in our laboratory.

Data Analysis - The proportions of species that could be identified in each of the three sites were compared using a chi square contingency test. To assess whether the unidentified species corresponded to less abundant species, the abundances of identified and unidentified species were compared in each area using a two-tailed Mann-Whitney test, with correction for continuity and for presence of tied ranks (Zarr 1999). To describe the effect of sample size on the proportion of unidentified species, two species accumulation curves were produced, using the "EstimateS" program (Colwell 1997). One curve included all species and the other excluded the identified species. Each point on the curves represented the average number of species in 50 subsamples of a specific size taken at random, without replacement. The curve corresponding to the proportion of unidentified species was calculated by dividing the results of the curve of unidentified species by that of all species together. In this analysis, a sample unit was defined as the flies caught in one trap on one day. A total of 944 units was examined.

Results and Discussion

A total of 29,289 *Drosophila* males were collected and identified. One hundred and twenty five species were found: 57 from Japi, 76 from I. Bela, and 90 from B. Rico. Seventy two of these were identified as described species, based on figures of the male genitalia found in the literature, and/or with the help of Professor Carlos R. Vilela. Table 1 shows the total number of males per species and the locality, as well as the references used for identification. Eight

Table 1. Total number of males per species and locality. The numbers following the name of the each species corresponds to the references used in identification. In the numbers underlined the figure used represented a specimen from the type series. "Vilela, personal com.", means that the identification was made by the first author based on drawings and explanations kindly provided by Prof. Carlos Vilela. The species underlined were not included in a previous list from São Paulo state.

Subgenus	Group	Species	Japi	B. Rico	I. Bela	Total
Drosophila	*annulimana*	*D. ararama* Pavan & Cunha (2)		2		2
		D. annulimana Duda (2, 26)	38	1	55	94
		D. arapuan Cunha & Pavan (2)	1		2	3
		D. aragua Vilela & Pereira (24)		12		12
	calloptera	*D. atrata* Burla & Pavan (22)		265	13	278
		D. quadrum Wiedemann (22)	1	8		9
		D. schildi Malloch (22)		2		2
	canalinea	*D. canalinea* Patterson & Mainland (22)		5		5
		D. albomarginata Duda (22)			7	7
		D. sp5	51			51
		D. sp7	28	696	11	735
		D. sp42	1	39	6	46
		D. sp73		1		1
		D. sp81		1		1
		D. spb3			4	4
		D. spb11			3	3
		D. spb30			1	1
		D. spb35			1	1
	cardini	*D. cardini* Sturtevant (11, 28)		11		11
		D. neocardini Streisinger (11, 28)		25	116	141
		D. polymorpha Dobzhansky & Pavan (11, 28)	22	127	7	156
	coffeata	*D. fuscolineata* Duda (22, 16 as *D. fumosa*)	1	27	22	50
		D. sp52 aff. *D. coffeata* Williston (19)		2	205	207
	dreyfusi	*D. briegeri* Pavan & Breuer (3)	59	43	159	261
		D. dreyfusi Dobzhansky & Pavan (3)	41	1	34	76
		D. krugi Pavan & Breuer (3)	6		48	54
	guarani	*D. griseolineata* Duda (22)	48	2033	9	2090
		D. guaru Dobzhansky & Pavan (22)		151	2	153
		D. maculifrons Duda (22)	23	189		212
		D. ornatifrons Duda (22, 16 as *D. guarani*)	60	20		80
		D. sp31	6	1	11	18
		D. sp67		1		1
	immigrans	*D. immigrans* Sturtevant (external morphology)	3	7		10
	mesophragmatica	*D.* sp61		2	7	9
		D. spb26			1	1

Table 1 (cont.)

Subgenus	Group	Species	Japi	B. Rico	I. Bela	Total
	pallidipennis	*D. pallidipennis* Dobzhansky & Pavan (11, 22)		2	12	14
	repleta	*D. buzzatii* Patterson & Wheeler (18, 4)		1		1
		D. fascioloides Dobzhansky & Pavan (18)	5	1	11	17
		D. ivai Vilela (18)		1		1
		D. mercatorum Patterson & Wheeler (18, 4)		3		3
		D. nigricruria Patterson & Mainland (18)		1		1
		D. onca Dobzhansky & Pavan (18)	16	544	166	726
		D. paranaensis Dreyfus & Barros (18, 4)		19		19
		D. pictilis Wasserman (18)			5	5
		D. pictura Wasserman (18)			9	9
		D. querubimae Vilela (18)		2		2
		D. repleta Wollaston (18)		1	3	4
		D. senei Vilela (18)	5	1		6
		D. sp8	10	110	17	137
		D. sp70 aff. *D. vicentinae* Vilela (18)		1		1
		D. sp74 aff. *D. aldrichi* Patterson & Crow (18, 7, 4)		1		1
		D. sp79 aff. *D. ivai* (18)		1	2	3
		D. spb5			91	91
		D. spb6			156	156
		D. spb32			3	3
		D. spb38			2	2
	tripunctata	*D. bandeirantorum* Dobzhansky & Pavan (20)	23	40	14	77
		D. bifilum Frota-Pessoa (Vilela, personal com.)	6	127	6	139
		D. cuaso Bächli, Vilela & Ratcov (1)	47	12	99	158
		D. frotapessoai Vilela & Bächli (22)		1		1
		D. medioimpressa Frota-Pessoa (20, 8)	4	3		7
		D. mediopicta Frota-Pessoa (20, 8)	120	13	6	139
		D. mediopunctata Dobzhansky & Pavan (4, 8)	177	356	7	540
		D. mediosignata Dobzhansky & Pavan (1)	44	2		46
		D. mediostriata Duda (22, 4 as *D. crocina*)	25	107	1	133
		D. mesostigma Frota-Pessoa (16, 20)		1		1
		D. neoguaramunu Frydenberg (22)		2	4	6
		D. nigricincta Frota-Pessoa (16)			4	4
		D. paraguayensis Duda (1)	926	3656	4	4586
		D. paramediostriata Townsend & Wheeler (25)	3	384		387
		D. roehrae Pipkin & Heed (20, 13)	51	42	1	94
		D. setula Heed & Wheeler (27)	1		1	2
		D. spinatermina Heed & Wheeler (27)		90		90
		D. trapeza Heed & Wheeler (27, 12 as *D. mirassolensis*)	3	64		67
		D. trifilum Frota-Pessoa (8)	23	539	6	568

Table 1 (cont.)

Subgenus	Group	Species	Japi	B. Rico	I. Bela	Total
		D. sp.1 *D. angustibucca* sensu Frota-Pessoa, 1954; non Duda, 1925 (8, Vilela, personal com.)	105	92		197
		D. sp17	3			3
		D. sp18	1		4	5
		D. sp22 aff. *D.* sp1	29	7	1	37
		D. sp28	11	797	11	819
		D. sp33	3	25	3	31
		D. sp37	8		1	9
		D. sp38	1			1
		D. sp50		4	177	181
		D. sp76		1		1
		D. spb12			13	13
		D. spb16			1	1
		D. spb25			2	2
		D. spb27			2	2
		D. spb36		1	1	2
		D. spb37			1	1
	ungrouped	*D. caponei* Pavan & Cunha (16, 21)		39	52	91
		D. sticta Wheeler (Vilela, personal com.)		1		1
		D. spb13 aff. *D. caponei*			2	2
Siphlodora	ungrouped	*D. flexa* Loew (24)		1		1
Sophophora	*melanogaster*	*D. malerkotliana* Parshad & Paika (17)	11	258	68	337
		D. melanogaster Meigan (14)		4		4
		D. simulans Sturtevant (14)	6	171	28	205
	saltans	*D. austrosaltans* Spassky (9)		4		4
		D. neoelliptica Pavan &Magalhães (9)	1		28	29
		D. neosaltans Pavan & Magalhães (9)			22	22
		D. prosaltans Duda (9)		226	23	249
		D. sturtevanti Duda (9)	151	2160	116	2427
	willistoni	*D. bocainensis* Pavan & Cunha (10, 29)	19	9		28
		D. bocainoides Carson (29)		2		2
		D. capricorni Dobzhanzky & Pavan (10)	495	84	422	1001
		D. changuinolae Wheeler & Magalhães (29)		3		3
		D. fumipennis Duda (10, 22)	35	112	324	471
		D. nebulosa Sturtevant (10)	14	137		151
		D. paulistorum Dobzhanzky & Pavan (5, 10, 6, 15)	309	804	1707	2820
		D. willistoni Sturtevant (5, 10, 15, 22)	925	5632	479	7036
unidentified		*D.* sp10	5		19	24
		D. sp12	1			1
		D. sp26	4	2	7	13
		D. sp34	2	1		3
		D. sp41	1			1
		D. sp53		3		3
		D. sp55		1		1
		D. sp64		3		3
		D. sp66		3	7	10
		D. sp77		1		1

Table 1 (cont.)

Subgenus	Group	Specie	Japi	B. Rico	I. Bela	Total
		D. sp82		1		1
		D. spb1			5	5
		D. spb21			1	1
		D. spb34			1	1
		Total of individuals	4018	20389	4882	29289

1. Bächli *et al*., 2000; 2. Breuer & Pavan, 1950; 3. Breuer & Pavan, 1954; 4. Breuer & Rocha, 1971; 5. Burla, *et al*., 1949; 6. Cordeiro, 1952; 7. Fontedevila *et al*., 1990; 8. Frota-Pessoa, 1954; 9. Magalhães & Björnberg, 1957; 10. Malogolowkin, 1952; 11. Malogolowkin, 1953; 12. Mourão & Gallo, 1967; 13. Pipkin & Heed, 1964; 14. Salles, 1948; 15. Spassky, 1957; 16. Val, 1982; 17. Val & Sene, 1980; 18. Vilela, 1983; 19. Vilela, 1984[a]; 20. Vilela, 1992; 21. Vilela, 2001; 22. Vilela, & Bächli, 1990; 23. Vilela, & Bächli, 2000; 24. Vilela & Pereira, 1982; 25. Vilela & Pereira, 1985; 26. Vilela & Val, 1983; 27. Vilela & Val, 1985; 28. Vilela *et al*., 2002; 29. Wheeler & Magalhães, 1962.

males of *D. latifasciaeformis* Duda 1940 were caught at B. Rico, but this species was not included in the list because, as recommended by Grimaldi (1990), the subgenus *Scaptodrosophila* (which includes this species) should be given the status of genus.

Besides the species shown in table 1, we also observed the occurrence of another species, *D. metzii* Sturtevant 1921 (*tripunctata* group), which has never been registered before in Brazil (Vilela 1984b). Several males of this species were collected over palm flowers and fruits in an urban park (Bosque dos Jequitibás) in Campinas city. The identification was based on a comparison with the drawing of the aedeagus of a holotype provided by Vilela (1984).

Tidon-Sklorz & Sene (1999) provided a list of 93 species recorded in São Paulo state. We found 15 species not cited in that list. Of these, one was described (*D. cuaso*) and one revalidated (*D. mediosignata*) by Bächli *et al.* (2000), who also reported the presence of these species in São Paulo after the publication of the paper by Tidon-Sklorz & Sene (1999).

One species in the previous list, *D. mirassolensis,* was synonymized with *D. trapeza* (also present in the list) by Vilela & Val (1985), and another one, *D. angustibucca*, in fact corresponds to a misidentification of an undescribed species (Goñi *et al.* 1998). If one accepts the elevation of the subgenus *Scaptodrosophila* to genus, them a total of 105 described species of *Drosophila* are registered in São Paulo state.

The numbers of species found in each site are among the highest in Brazilian inventories. This was an unexpected result, since previous inventories used procedures chosen to maximize the efficiency of species detection, without the limitation of the standardization of the collecting method or sampling design.

The main aim of this project was to study the spatial distribution of some species in response to an environmental gradient (manuscript in preparation). The restrictions necessary to study a spatial pattern, i.e., the use of only one type of bait, one kind of trap, and the inclusion of all sampling points to a homogeneous area (except for the studied gradient) could have limited the chance of detecting species. Consequently it could result on a smaller number of species in the lists than in lists produced with several collecting methods. On the contrary, our results indicate that samples obtained with a single well standardized collecting method can be used more efficiently in species inventories of *Drosophila*, with the additional advantage that it is possible to quantify sampling efforts.

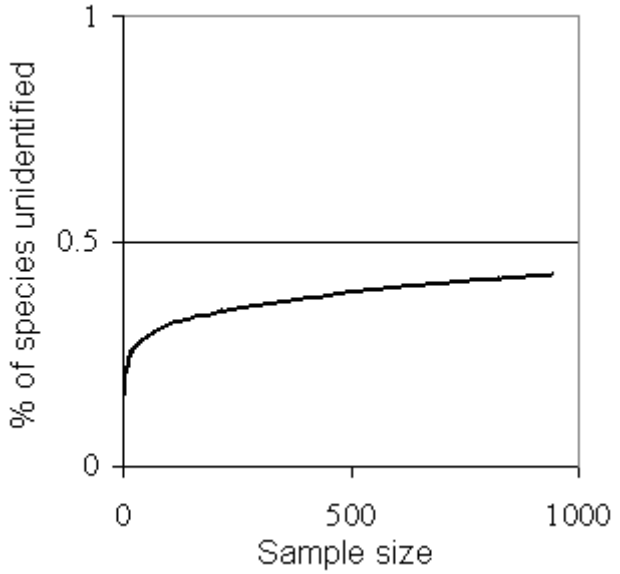
The high number of species found probably reflected the sensitivity of our identification method. Large proportion of an inventory usually consists of rare species, and many species are detected on the basis of only a few or even a single individual (Magurran 1988). This was the case in this study, since the proportion of species detected with $\leq$ 5 individuals was 61% in B. Rico, 37% in Japi and 48% in I. Bela . The chance of detecting a species also diminishes as the list of species already detected increases, because of the increasing probability of it resembling a species detected before. Vilela & Mori (1999) reported some species that were not detected in a previous inventory of the same region (Tidon-Sklorz *et al.* 1994). These authors suggested that such species may have been overlooked because they were very similar to more abundant species already present in the list. Vilela (2001) also suggested that the low occurrence of *D. caponei* in previous inventories reflects its frequent inclusion in an unidentified group. Maximum sensitivity is therefore essential for the method used to detect species in an inventory project. Our results indicate that the concentration of the identification effort on the male genitalia provides better results than when this criterion is applied only when doubts appear during identification based on external morphology, as is usually done.

Overall, we identified 72 species (58% of the total) a result similar to that obtained by Val & Kaneshiro (1988), who identified 50% of the 82 species detected. The proportion of identified species did not differed significantly between sites (67% for Japi, 71% for B. Rico, and 54% for I. Bela; $p=0.14$). Thus we have no evidence that these forest types are different regarding the proportion of species remaining to be described.

Some of our unidentified species have probably already been described. These species may not have been recognized because of the lack of detailed representation of the aedeagus in the literature, but they are probably a minority. Recently, an effort has been made to produce detailed figures of the aedeagus of most neotropical *Drosophila* (e.g. references in Table 1), with the *tripunctata* group receiving special attention. Vilela (1992) pointed out that there are still four species in this group for which the male genitalia are incompletely illustrated in the literature. One of these was present in our samples and was identified in collaboration with Prof. Carlos R. Vilela. However, 16 species of this group could not be identified. Even if one assumes that the unidentified species include all three species with unillustrated genitalia, there are least 13 undescribed species in this group. We therefore believe that the majority of the unidentified species are in fact undescribed.

The average number of individuals per species at the three sites was significantly greater for identified species (Japi, $p < 0.05$; B. Rico, $p < 0.001$; I. Bela, $p < 0.001$). On the total of the three sites, identified species were on average almost seven times more abundant than the unidentified ones. A lower abundance of unidentified species was expected because species which are rarer and/or less attracted to traditional baits have a lower probability of being studied. The same factors obviously influenced the probability

in figure 1, which illustrates how the proportion of unidentified species increases with sample size, without reaching a plateau in the sample studied. To this reasoning we must add that some *Drosophila* species are not attracted to banana baits at all, and these are the least studied taxonomicaly. Thus, it is reasonable to believe that a larger proportion of the total of described species has been detected, and suggests that our value of 58% of described species is an overestimate.



The combined use of standardized collecting procedures and a technique with a high and consistent sensitivity for species identification allows the use of methods to quantify biological diversity. This approach highlights the great diversity of *Drosophila* species in forest remnants in São Paulo state. Moreover, it indicates that at least about half of the species in this region remain to be described. The forests of southeastern Brazil, especially those of São Paulo state, are among the most studied ecosystems in this country (Lewinsohn & Prado 2002), including for *Drosophila* (Tidon-Sklorz & Sene 1999). *Drosophila* genus is also an exception between the mega diversified taxa of small forest dwelling invertebrates, by having received a significative taxonomic attention. Thus, our conclusions illustrate the actual state of knowledge about Brazilian biota, from which the proportion of described species is estimated as one tenth (Lewinsohn & Prado 2002). Moreover, it shows that *Drosophila* genus still needs intensive taxonomic effort as the most of our taxa. A conclusion that becomes more relevant considering that this is a well studied genus, on the best sampled region of Brazil.

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