Drosophilids (Diptera) from an Atlantic Forest Area in Santa Catarina, Southern Brazil

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ABSTRACT. Drosophilids (Diptera) from an Atlantic Forest Area in Santa Catarina, Southern Brazil. The present work aims at knowing the faunal composition of drosophilids in forest areas of southern Brazil. Besides, estimation of species richness for this fauna is briefly discussed. The sampling were carried out in three well-preserved areas of the Atlantic Rain Forest in the State of Santa Catarina. In this study, 136,931 specimens were captured and 96.6% of them were identified in the specific level. The observed species richness (153 species) is the largest that has been registered in faunal inventories conducted in Brazil. Sixty-three of the captured species did not fit to the available descriptions, and we believe that most of them are non-described species. The incidence-based estimators tended to give rise to the largest richness estimates while the abundance based give rise to the smallest ones. Such estimators suggest the presence from 172.28 to 220.65 species in the studied area. Based on these values, from 69.35 to 88.81% of the expected species richness were sampled. We suggest that the large richness recorded in this study is a consequence of the large sampling effort, the capture method, recent advances in the taxonomy of drosophilids, the high preservation level and the large extension of the sampled fragment and the high complexity of the Atlantic Rain forest. Finally, our data set suggest that the employment of estimators of richness for drosophilid assemblages is useful but it requires caution.

KEYWORDS. Atlantic Rain Forest; *Drosophila*; Neotropic; species richness estimation; taxonomic survey.

RESUMO. Drosofilídeos (Diptera) de uma Área de Floresta Atlântica em Santa Catarina, Sul do Brasil. O presente estudo tem como objetivo conhecer a composição da fauna de drosofilídeos em áreas de floresta no sul do Brasil. Além disso, a estimativa da riqueza de espécies desta fauna é brevemente discutida. As amostras foram realizadas em três áreas bem preservadas da Mata Atlântica no estado de Santa Catarina. Neste estudo, 136.931 espécimes foram capturados e 96,6% destes foram identificados em nível específico. A riqueza de espécies observada (153 espécies) é a maior já registrada em inventários faunísticos realizados no Brasil. Dentre as espécies capturadas, sessenta e três não se adequaram às descrições disponíveis e a maioria destas provavelmente não foi descrita. Os estimadores baseados em incidência de espécies tenderam a gerar as mais altas estimativas de riqueza enquanto aqueles baseados em abundância geraram as menores. Tais estimadores sugerem a presença de 172,28 a 220,65 espécies na área estudada. Baseando-se nestes valores, de 69,35 a 88,81% da riqueza de espécies esperada foi amostrada. Sugere-se que a alta riqueza registrada neste estudo é uma conseqüência do grande esforço amostral, do método de captura, de recentes avanços na taxonomia de drosofilídeos, do alto grau de preservação e ampla extensão do fragmento amostrado e da alta complexidade da Mata Atlântica. Finalmente, os dados obtidos sugerem que a aplicabilidade de estimadores de riqueza para assembléias de drosofilídeos é útil, mas requer cautela.

PALAVRAS-CHAVE. *Drosophila*; estimativa da riqueza de espécies; levantamento taxonômico; Mata Atlântica; Neotrópico.

The Drosophilidae family of flies includes 73 genera and 3,938 described species, many of them (1,148) belonging to the genus *Drosophila* Fallen 1823 (Bächli 2006). In Brazil, 18 genera and 304 species have been registered, mainly of the genera *Drosophila* and *Zygothrica* Wiedemann 1830 (180 and 54 species, respectively) (Gottschalk et al., manuscript in preparation).

Several drosophilid species can coexist, what gives rise to very complex systems, mainly in the tropics (Dobzhansky & Pavan 1950). Most of these flies feed on microorganisms, especially yeasts, associated with decaying fruits and fungi. While some of these species use one or few feeding and breeding sites, another are more versatile and use a wider range of resources (Cunha 1957; Cunha et al. 1957; Begon 1982).

Nowadays, the interest in drosophilids as models in biodiversity distribution studies and their causes are increasing (e.g. Sevenster & van Alphen 1993; Shorrocks & Sevenster 1995; Worthen et al. 1998). For this reason, conducting inventories of drosophilids becomes very important, since, except for a few cases, the knowledge about their distributions in the Neotropics is not enough for a discussion concerning this issue (Val et al. 1981). Likewise, inventories supply useful information for the detection of tendencies, impacts or recovery of ecosystems. This information allows for the selection and maintenance of conservation areas and also the understanding of primary environmental factors that control the species richness (Chao 2005). These data become still more important facing to the current picture of fragmentation in native ecosystems, since this threat strongly affects ecological and evolutionary processes (Terborgh 1992; Laurance 1997).

The Atlantic Rain Forest is biologically one of the more complex and speciose natural systems all over the world, with about 7% of the richness of the Earth (Quintela 1990). However, only 7% of its original range remains preserved, what turns it one of the more threatened biomes of the planet. Its high biodiversity and the threat status on this area make the Atlantic Rain Forest a biodiversity hotspot, i.e., one of the areas of conservation priority. Thus, to characterize its biota and to understand the processes of it in this biome should be a priority (Myers et al. 2000).

In this article, we aim to know the faunal composition of drosophilids in an area of the Atlantic Rain Forest in southern Brazil and compare the observed richnes with that of another regions and ecosystems in South America. Besides, we compare the observed richness with the expected one and discuss the use of species richness estimators for drosophilid assemblages.

MATERIAL AND METHODS

Collections of drosophilids were conducted in the municipality of Joinville, State of Santa Catarina, southern Brazil. This city is located in the limit zone of the annual isotherm of 20ºC in the Neotropics and its climate is Cfa according to Koeppen classification. The annual rainfall rate exceeds 2,100mm and the average annual relative humidity is about 70% (Prefeitura Municipal de Joinville/ Planisul S.A. 1975). Along the sampled period, the average temperature and average relative humidity were 22.72ºC and 79.39%, respectively, while the average annual rainfall rate was 1,849.96mm (these data are a courtesy of the Universidade da Região de Joinville – UNIVILLE).

The studied area (26°17'37,9''S; 49°00'56,4''W), known as Piraí, is located in the southernmost area of the Serra do Mar. Piraí is covered by a well-preserved vegetation (Atlantic Rain Forest) and it is subjected to a very low human influence.

Three sites in a contiguous forest, with quite similar vegetation and geomorphology, were sampled: P1, P2 and P3. P1 and P2 are located in a conservation area with restricted access and are 200m far from each other. The first of them is situated in the margin of Piraí River, about 30m far from the border of the forest fragment. P2 is located at the base of a hill and it is 75m far from the border of the forest fragment. In P3, 4.5km far from the others, the human influence is still reduced, but it is higher than in P1 and P2 (there are small agricultural and livestock areas and some inhabitants).

For the drosophilid collection, traps based on Tidon & Sene (1988) with kneaded banana bait (100g on average per trap) and yeast were used. These traps stayed during three days in the field, knotted to trees and about 1.5m above the ground.

In P1 and in P2, 25 traps were distributed in areas of about 200m^2 per collection. In P3, the traps were placed every 50m in a 500m transect, from the forest fragment border to the core, totaling 11 subsites and also in two subsites in the matrix of such fragment (0.9 and 1.5km far from its border, respectively) with a higher human influence. In P3, six traps were used per subsite, totaling 78 traps per collection. The placement of the traps in P1 and P2 and in the subsites of P3 was arbitrary and made according to the availability of trees. Sixteen collections were carried out in P1 and in P2, while eight collections were conducted in P3 (Table I).

Identification of specimens was based on external morphology. With this purpose, identification keys and descriptions and redescriptions of species were used. Very similar or sibling species were distinguished either by the analysis of male genitalia without its removal, according to Spassky (1957), or by dissection and preparation according to Wheeler & Kambysellis (1966). Females of such species were identified by the genitalia of the male offspring, when it was possible. For a wide list of references on identification, see Medeiros & Klaczko (2004) and Bächli (2006).

The flies of the subgroup *willistoni* were not identified at the specific level due to the high difficulty to differentiate its species and the large number of collected specimens, being considered here as having together the status of species. Only two species of it, *Drosophila paulistorum* Dobzhansky & Pavan 1949 and *D. willistoni* Sturtevant 1916, have been registered in southern Brazil. Studies in this area have suggested that *D. willistoni* is, by far, much more abundant than *D. paulistorum*. The few specimens of such subgroup dissected in our study were all assigned to *D. willistoni*.

Voucher specimens were pinned (double-mounted) and deposited in the Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre (RS).

Table I. Dates of traps removal of each collection in the sampled sites P1, P2 and P3 of Piraí.

Season and year	P1/P2	P3
Spring 2001	09 Oct.	-
Summer 2002	22 Jan.	-
Autumn 2002	19 May	-
Winter 2002	21 Jul.	-
Spring 2002	03 Nov.	-
Summer 2003	24 Feb.	-
Autumn 2003	04 May	-
Winter 2003	27 Jul.	-
Spring 2003	18 Nov.	02 Dec.
Summer 2004	25 Jan.	23 Feb.
Autumn 2004	18 May	17 May
Winter 2004	06 Aug.	05 and 09 Aug.
Spring 2004	04 Nov.	03 Nov.
Summer 2005	28 Feb.	27 Feb.
Autumn 2005	11 May	10 May
Winter 2005	20 Aug.	-

Additional material is kept in microvials with ethanol 70%, or glycerol (for dissected terminalias), in the Laboratório de *Drosophila* (UFRGS).

Species richness estimates for each site and for the studied area (Piraí) were obtained through the software EstimateS Version 8 (Colwell 2006). For the sites P1, P2 and P3, the sample unit was the collection, i.e., sixteen sample units were acconted for P1 and P2 and 8 for P3. To estimate the species richness for Piraí we used the data obtained in P1, P2 and P3. In this last case, though, each sample in P3 was subdivided in three subsamples in order to level the sampling effort (number of traps and size of sampled area) to the sampling effort in P1 and P2. These subsamples were composed by a - matrix and subsites between 0 and 50m from the border in the transect; b – subsites between 100 and 250m from the border; and c - subsites between 300 and 500m from the border. The equivalence of sampling effort is a constraint to the application of the non-parametric estimators used: ACE, ICE, Chao 1, Chao 2, Jacknife 1, Jacknife 2, Bootstrap and Michaelis-Menten (MM from now on). The number of randomizations, if the estimator requires it, was 1,000.

RESULTS

A total of 136,931 drosophilid flies were captured, of which 132,259 (96.6%) were identified at the specific level, 4,595 at the species group level (of the genus *Drosophila*) and 77 at the genus level (38 specimens of *Drosophila* and 39 of *Zygothrica*). Among such specimens, we found 153 species (103 in P1, 105 in P2 and 112 in P3), of which 133 were assigned to the genus *Drosophila*, 13 to the *Zygothrica*, two to the *Diathoneura* Duda 1924 and one to the *Amiota* Loew 1862, *Cladochaeta* Coquillett 1900, *Neotanygastrella* Duda 1925, *Zaprionus* Coquillett 1901 and *Scaptodrosophila* Duda 1923 (Table II). Only nine exotic species (but 8.01% of the total number of specimens) were registered, seven belonging to the genus *Drosophila* (*D. ananassae*, *D. busckii*, *D. immigrans*, *D. malerkotliana*, *D. melanogaster*, *D. simulans*, and *D.* sp.ml1 – of the group *melanogaster*), one to the *Zaprionus* (*Z. indianus*) and one to the *Scaptodrosophila* (*S. latifasciaeformis*).

The species richness estimators suggested a number from 172.28 (Bootstrap) to 220.65 (Jacknife 2) species in the regional pool (Piraí) (MM suggested 151.09 species, but this number is lower than the observed species richness, 153). In P1, the richness was estimated between 117.02 (Bootstrap) and 149.75 species (Jacknife 2), while in P2 it varied between 118.67 (MM) and 168.70 (Jacknife 2). Finally, the lowest estimate obtained in P3 was 128.05 (Bootstrap) and the highest was 171.25 (Jacknife 2) (Fig. 1).

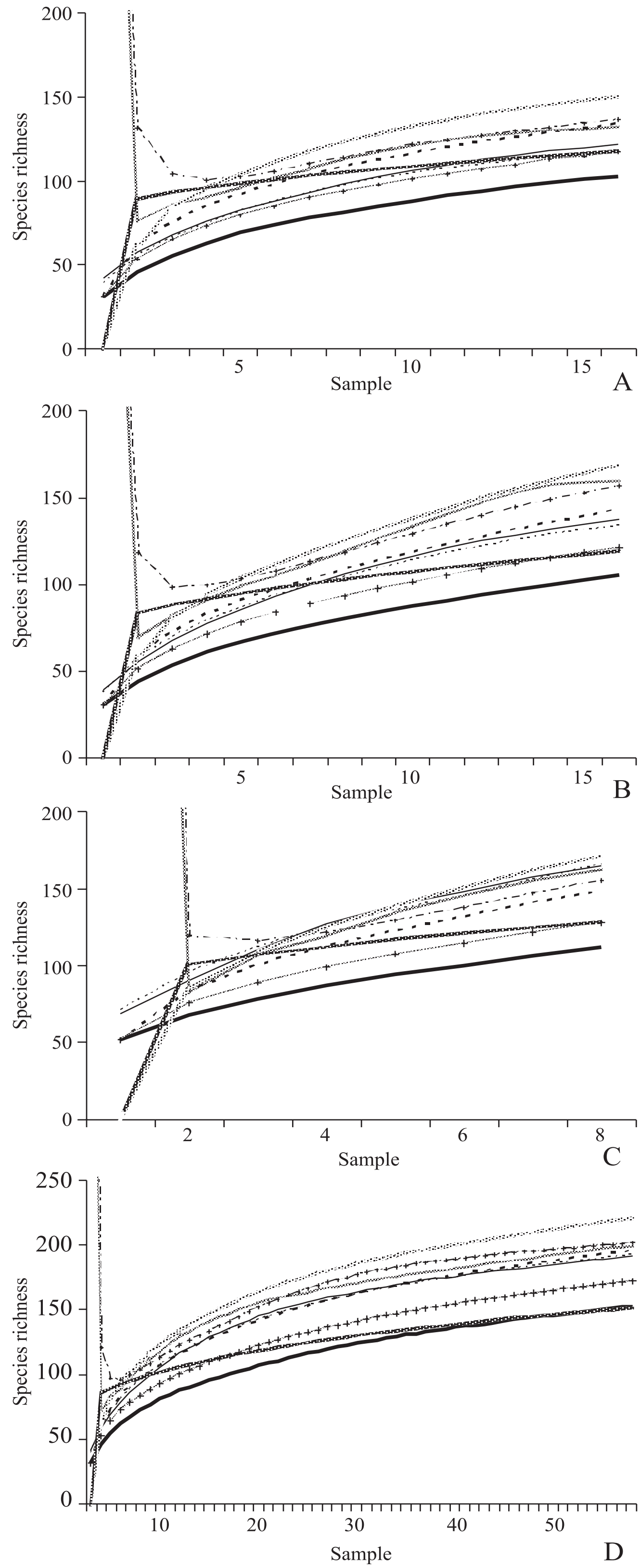

Fig. 1. Estimated species accumulation curves and species accumulation curves (randomized) in (A) P1, (B) P2 and (C) P3 and (D) in the studied area (Piraí). ——— ACE, — —+— — ICE, - - - - - - - Chao 1, ~~~~~ Chao 2, ▬ ▬ ▪ Jacknife 1, ▒▒▒ Jacknife 2, ·····+····· Bootstrap, ▫▫▫▫▫ MM, ▬▬▬ species accumulation curve (randomized).

DISCUSSION

The comparison between the richness observed in Piraí and those detected in other studied areas in Brazil is limited because the collection methods are not standardized (methods

Table II. Absolute abundances of the drosophilid species collected in Piraí (Joinville, southern Brazil) in the sites P1, P2 and P3.

Genus	Species group	Species	Absolute abundance			
			P1	P2	P3	Total
Amiota	unidentified	*A.* sp.am1	-	-	1	1
Cladochaeta	-	*C.* sp.cl1	1	-	-	1
Drosophila	*annulimana*	*D. annulimana* Duda, 1927	16	19	91	126
		D. ararama Pavan & Cunha, 1947	-	-	4	4
		D. arassari Cunha & Frota-Pessoa, 1947	1	-	1	2
		unidentified[A]	2	1	4	7
	bromelioides	*D. bromelioides* Pavan & Cunha, 1947	-	1	-	1
	busckii	*D. busckii* Coquillett, 1901*	2	-	15	17
	calloptera	*D. atrata* Burla & Pavan, 1953	4	3	2	9
		D. calloptera Schiner, 1868	3	-	2	5
		D. quadrum (Wiedemann, 1830)	-	-	2	2
	canalinea	*D. albomarginata* Duda, 1927[B]	2	7	1	10
		D. davidgrimaldii Vilela & Bächli, 1990[B]	1	-	2	3
		D. sp.cn1	7	-	-	7
		D. sp.cn2	-	1	-	1
		D. sp.cn3	-	-	1	1
		D. sp.cn4	-	-	1	1
		D. sampa Ratcov & Vilela, 2007	5	2	1	8
		D. piratiniga Ratcov & Vilela, 2007	11	37	3	51
		D. sp.cn7	-	3	-	3
		unidentified[A]	3	11	2	16
		D. caponei Pavan & Cunha, 1947	49	57	1122	1228
	cardini	*D. cardini* Sturtevant, 1916	-	4	10	14
		D. cardinoides Dobzhansky & Pavan, 1943	11	17	4	32
		D. neocardini Streisinger, 1946	47	23	43	113
		D. neomorpha Heed & Wheeler, 1957#	418	172	1522	2112
		D. polymorpha Dobzhansky & Pavan, 1943	1071	626	1516	3213
		D. sp.cd1	-	2	3	5
		unidentified[A]	-	-	3	3
	coffeata	*D. coffeata* Williston, 1896	234	266	91	591
		D. fuscolineata Duda, 1925	12	10	31	53
		unidentified[A]	3	1	-	4
	dreyfusi	*D. briegeri* Pavan & Breuer, 1954	17	18	8	43
		D. dreyfusi Dobzhansky & Pavan, 1943	15	39	15	69
		D. krugi Pavan & Breuer, 1954[B]	-	3	-	3
		unidentified[A]	5	12	1	18
	guarani	*D. griseolineata* Duda, 1927	187	113	137	437
		D. guaraja King, 1947	111	46	115	272
		D. guaru Dobzhansky & Pavan, 1943	-	4	66	70
		D. maculifrons Duda, 1927	1	-	3	4
		D. ornatifrons Duda, 1927	15	-	48	63
		D. sp.gu1	4	1	14	19
		D. sp.gu2	93	45	64	202
		D. sp.gu3	-	1	-	1
		D. sp.gu4	2	-	1	3
		D. sp.gu5	-	1	1	2
		unidentified[A]	20	10	-	30
	immigrans	*D. immigrans* Sturtevant, 1921*	29	33	50	112
	melanogaster	*D. ananassae* Doleschall, 1858*	1	1	2	4
		D. malerkotliana Parshad & Paika, 1964*	1179	317	2883	4379
		D. melanogaster Meigen, 1830*	232	41	1	274
		D. simulans Sturtevant, 1919*	1594	750	3143	5487
		D. sp.ml1*	-	1	-	1
	morelia	*D. carioca* Vilela & Bächli, 2004[B]	2	1	-	3
		D. fluminensis Vilela & Bächli, 2004[B]	1	2	46	49
		D. morelia Vilela & Bächli, 2004[B,C]	25	83	74	182
	pallidipennis	*D. pallidipennis* Dobzhansky & Pavan, 1943	16	17	33	66
	peruensis	*D. boraceia* Vilela & Val, 2004#	-	-	1	1

Table II. Cont.

Genus	Species group	Species	Absolute abundance			
			P1	P2	P3	Total
	repleta	*D. carolinae* Vilela, 1983	29	28	28	85
		D. ellisoni Vilela, 1983	10	5	21	36
		D. fascioloides Dobzhansky & Pavan, 1943	57	70	299	426
		D. hydei Sturtevant, 1921	5	6	41	52
		D. mapiriensis Vilela & Bächli, 1990	-	-	1	1
		D. mercatorum Patterson & Wheeler, 1942	173	70	698	941
		D. meridionalis Wasserman, 1962	-	2	1	3
		D. nigricruria Patterson & Mainland, 1943	-	-	1	1
		D. onca Dobzhansky & Pavan, 1943	52	51	53	156
		D. pictura Wasserman, 1962#	8	6	1	15
		D. repleta Wollaston, 1858	1	-	1	2
		D. zottii Vilela, 1983	3	2	2	7
		D. sp.re1	1	-	-	1
		D. sp.re2	11	-	-	11
		D. sp.re3	227	202	123	552
		D. sp.re4	2	-	1	3
		unidentified[A]	215	197	666	1078
	saltans	*D. neoelliptica* Pavan & Magalhaes, 1950	83	74	314	471
		D. neosaltans Pavan & Magalhaes, 1950	4	13	15	32
		D. prosaltans Duda, 1927	68	22	91	181
		D. pulchella Sturtevant, 1916	1	1	-	2
		D. saltans Sturtevant, 1916	1	2	4	7
		D. sturtevanti Duda, 1927	4833	3286	7087	15206
		D. sp.sa1	3	2	1	6
		unidentified[A]	1306	711	29	2046
	tripunctata	*D. bandeirantorum* Dobzhansky & Pavan, 1943	114	141	238	493
		D. bifilum Frota-Pessoa, 1954#	1	-	1	2
		D. bipunctata Patterson & Mainland, 1943#	-	1	1	2
		D. cuaso Bächli, Vilela & Ratcov, 2000	136	79	93	308
		D. medioimpressa Frota-Pessoa, 1954	5	-	-	5
		D. mediopicta Frota-Pessoa, 1954	643	600	820	2063
		D. mediopunctata Dobzhansky & Pavan, 1943	76	85	210	371
		D. nappae Vilela, Valente & Basso-da-Silva, 2004	37	24	47	108
		D. neoguaramunu Frydenberg, 1956	9	4	-	13
		D. paraguayensis Duda, 1927	968	1339	1285	3592
		D. paramediostriata Townsend & Wheeler, 1955	9	6	1	16
		D. roehrae Pipkin & Heed, 1964	37	50	135	222
		D. setula Heed & Wheeler, 1957	7	15	1	23
		D. trifilum Frota-Pessoa, 1954	3	3	1	7
		D. tripunctata Loew, 1862	1	-	-	1
		D. unipunctata Patterson & Mainland, 1943	12	1	7	20
		D. sp.tp1	2	-	-	2
		D. sp.tp2	3	-	-	3
		D. sp.tp3	1	-	-	1
		D. sp.tp4	1	1	-	2
		D. sp.tp5	-	3	1	4
		D. sp.tp6	2	-	2	4
		D. sp.tp7	-	-	1	1
		D. sp.tp8	4	2	4	10
		D. sp.tp9	4	12	2	18
		D. sp.tp10	1	-	-	1
		unidentified[A]	512	752	129	1393
	willistoni	*D. bocainoides* Carson, 1954	-	1	-	1
		D. capricorni Dobzhansky & Pavan, 1943	6664	7478	9385	23527
		D. fumipennis Duda, 1925	1111	1597	2421	5129
		D. nebulosa Sturtevant, 1916	2	1	1	4
		D. parabocainensis Carson, 1954[B]	9	4	16	29
		D. sgr. *willistoni* Pavan, 1952	18492	15794	21965	56251

Table II. Cont.

Genus	Species group	Species	Absolute abundance			
			P1	P2	P3	Total
	ungrouped	*D. flexa* Loew, 1866	1	1	6	8
	unidentified	*D.* sp.ob1	-	6	-	6
		D. sp.ob2	3	-	3	6
		D. sp.ms1	66	72	57	195
		D. sp.ms2	-	1	-	1
		D. sp.bo1	-	-	1	1
		D. sp.bo2	-	-	1	1
		D. sp.bo3	-	-	1	1
		D. sp.as1	-	-	2	2
		D. sp.as2	40	41	66	147
		D. sp.as3	16	16	35	67
		D. sp.as4	2	-	4	6
		D. sp.dr1	-	1	-	1
		D. sp.dr2	39	37	155	231
		D. sp.dr3	222	88	711	1021
		D. sp.dr4	6	4	4	14
		D. sp.dr5	-	-	6	6
		D. sp.dr6	-	-	1	1
		D. sp.dr7	-	-	1	1
		D. sp.dr8	1	-	1	2
		D. sp.dr9	6	7	3	16
		D. sp.dr10	-	1	-	1
		D. sp.dr11	-	-	1	1
		D. sp.dr12	-	2	-	2
		D. sp.dr13	-	1	-	1
		D. sp.dr14	1	-	-	1
		D. sp.dr15	-	1	-	1
		D. sp.dr16	-	-	1	1
		unidentified[A]	10	2	26	38
Diathoneura	-	*D.* sp.dt1	-	1	-	1
	-	*D.* sp.dt2	-	1	2	3
Neotanygastrella	-	*N.* sp.nt1	-	-	1	1
Scaptodrosophila	*latifascieformis*	*S. latifascieformis* (Duda, 1940)*	2	2	419	423
Zaprionus	*armatus*	*Z. indianus* Gupta, 1970*	25	4	242	271
Zygothrica	*atriangula*	*Z. poeyi* (Sturtevant, 1921)#	3	1	-	4
	bilineata	*Z. bilineata* (Williston, 1896)	-	3	-	3
	dispar	*Z. dispar* (Wiedemann, 1830)	-	1	-	1
		Z. nigropleura Grimaldi, 1987#	-	1	-	1
	hypandriata	*Z. hypandriata* Burla, 1956#	-	1	-	1
		Z. lanceolata Burla, 1956#	-	2	-	2
	orbitalis	*Z. orbitalis* (Sturtevant, 1916)	-	-	5	5
	vittimaculosa	*Z. vittimaculosa* Burla, 1956	2	1	-	3
	ungrouped	*Z. apopoeyi* Burla, 1956#	-	2	-	2
	unidentified	*Z.* sp.zy1	-	1	-	1
		Z. sp.zy2	1	-	1	2
		Z. sp.zy3	1	-	-	1
		Z. sp.zy4	2	-	-	2
		unidentified[A]	13	22	4	39
		Total	41873	35869	59189	136931

([A]) "unidentified" species do not include those recognized as species but not identified. New register ([B]) in the State of Santa Catarina and in southern Brazil and ([C]) in South America. * exotic species. # In southern Brazil, those species were just collected in the studied area (Piraí) but their occurrences were registered in previous papers (De Toni et al. 2005; Döge et al. 2006, 2007; Gottschalk et al. 2006).

of attraction and capture, sampled area, etc.). However, the number of species observed in the present study stands out, since our data set represents the largest species richness registered in inventories carried out in Brazil.

The studies by Val & Kaneshiro (1988) and Medeiros & Klaczko (2004), conducted in southeastern Brazil, and that by Gottschalk et al. (2007), conducted 160km southward from Joinville, also stand out for the amount of observed species.

At the Estação Biológica de Boracéia – in primary forest (Atlantic forest), capoeira (intermediate successional stage of Atlantic Rain Forest recovery) and grassland with cultivated plants –, Val & Kaneshiro (1988) registered 152 species. Such authors used several types of bait and capture methods. Medeiros & Klaczko (2004) collected 125 species of *Drosophila* in three areas "which differ clearly in their climatic and geomorphological conditions": Atlantic Rain Forest (76 species), hillside forest (90) and altitudinal forest (57). Gottschalk et al. (2007) registered 105 species in four sites with different human influence: Atlantic Rain Forest (48 species), capoeira (84), orchard (66) and an area with high urbanization level (64). Gottschalk et al. (2007), Medeiros & Klaczko (2004) and our study used the same bait and a very similar capture method.

Other studies concerning drosophilid inventories in Brazil did not detect richness larger than 70 species (Franck & Valente 1985; Valente & Araújo 1991; De Toni & Hofmann 1995; Saavedra et al. 1995; Vilela & Mori 1999; Schmitz et al. 2007; and others). However, these studies, in general, exhibit a smaller sampling effort and many specimens were not identified at the specific level.

Reasons for the large richness observed. The large richness in our study is probably a consequence of several factors:

a. Sampling effort. Up to now, the present study is the longest inventory conducted in Brazil (four years) and, to our knowledge, it presents the largest number of collections per site (in P1 and P2). In addition, the estimated species accumulation curves (Fig. 1) suggest that a larger number of species would be observed if our study was extended.

b. The capture method. Similarly to the present study, recent studies have been using traps that catch the flies attracted by bait, mainly banana. This method has been providing a larger number of collected specimens, as well as larger species richness (Tidon & Sene 1988; Medeiros & Klaczko 1999; Gottschalk et al. 2003).

c. Advances in the taxonomy of drosophilids. Formerly, identification and descriptions of drosophilid species were based on external morphology. Now, however, descriptions and redescriptions of species of Drosophilidae have been emphasizing the external and internal morphology of male terminalia (Vilela 1983), since it was observed that many species can be morphologically distinguished only by the analysis of such structure (Vilela 1992). Encouraged by this technique, a large number of descriptions of new species and descriptions of the terminalias has been published.

d. Preservation level and extension of the sampled fragment. The high level of preservation of the sampled area and the large extension of this fragment, one of the less fragmented Atlantic forest areas in southern Brazil (Fundação SOS Mata Atlântica 2005), should highly contribute on the observed richness. Fernández (2000) found that the diversity is directly proportional to the area of the fragment, though this correlation is not linear. On the other hand, degradation of natural areas facilitate the introduction of invasive species -– both exotic and native ruderal -– (Martins 2001; Ferreira & Tidon 2005; Gottschalk et al. 2007) what can lead to a successional process (gradual qualitative and quantitative change in the community structure) and to a significant reduction in the species richness.

e. The complexity of the Atlantic Rain forest. Finally, the biome where our study was conducted was probably decisive. Several studies (Val & Kaneshiro 1988; Medeiros & Klaczko 2004; Schmitz et al. 2007; Gottschalk et al. 2007) suggest that the Atlantic forest (including its related ecosystems) is the richest for this fauna among the Brazilian biomes. Compared to other Brazilian biomes, the higher geomorphologic, vegetational and climatic heterogeneity along its whole extension seems to lead to this picture, though there is a larger number of studies in the Atlantic forest. In the whole biome Cerrado (and its related associated ecosystems, including urban), for instance, only 98 species of Drosophilidae were registered (Mata et al. 2008).

Non-described species. Another interesting aspect is that descriptions of species were extensively searched and evaluated, but 41.2% of the recognized species (54 species of the genus *Drosophila*, four of the *Zygothrica*, two of the *Diathoneura*, and one of the *Amiota*, *Cladochaeta*, *Neotanygastrella*) – 2,846 specimens (2.08% of the total) – did not fit to such descriptions. It is possible that some of these were not identified due to the inexistence of descriptions of the male terminalia, but we believe that most of them are non-described species. Similar situation was observed by Medeiros & Klaczko (2004) and Val & Kaneshiro (1988), who found, respectively, 42.4% and 50% of non-described species among the recognized ones.

The expected richness and the use of species richness estimators for drosophilid assemblages. Undoubtedly, the number of species observed in complex communities underestimates the true richness (Chao 2005). The use of species richness estimators has been proposed and developed to overcome such underestimation (Coleman 1981; Chao 1984, 1987; Palmer 1991; Chao et al. 1993; Lee & Chao 1994; Nichols et al. 1998). The efficiency of such estimators has been arduously discussed in scientific papers. However, analyses with distinct data sets indicate different richness estimators as the most efficient. For this reason, it has been suggested that the data set of each study determines which one is the best estimator (Palmer 1990; Coddington et al. 1996; Gotelli & Colwell 2001; Brose 2002).

In the present work, Bootstrap, an incidence-based species estimator, gave rise to the lowest estimates as well as in other studies (Brose 2002; Ganho & Marinoni 2005). Bootstrap is suggested as a very effective estimator by Palmer (1990), however, his data sets were obtained from quadrats, what avoid the possible implications of pseudoreplications. On the other hand, our samples were obtained along four years (time enough for significant changes in Drosophilidae assemblages composition), but in few sites.

The remaining incidence-based estimators – ICE, Chao 2, Jacknife1 and Jacknife 2 – tended to give rise to the largest richness estimates (Fig. 1). The large amount of uniques and duplicates (used in the formulas of such estimators) in our collections (about 50.0% of the collected species in P1, P2, P3 and in Piraí) seems to be the reason for this. Incidence-based species estimators are not appropriate to study assemblages with exceptionally large number of rare species (as observed in our data set) and, therefore, their use is restricted. Likewise, the use of Jacknife 1 is restricted because its formula just allows estimates that do not exceed the double of the number of collected species (Krebs 1999). Then, the use of such estimator is not recommended for samples very scattered along the time (like in our study) or for scarcely sampled areas. In all sites and Piraí, Jacknife 2 gave rise to the largest expected species richness, what is also observed by Carlton & Robinson (1998) in their study concerning the diversity of litter-dwelling beetles in an area in the USA.

Chazdon et al. (1998) analyze the influence of a non-random spatial distribution (patchiness) and sample size on the effectiveness of all the richness estimators used in our study. Such authors, comparing richness estimates of vegetational communities with the true number of species (obtained through exhausting inventories) of areas of Costa Rica, considered Chao 2 and ICE the most effective estimators. Besides, such work indicates that when Chao 2 and ICE failed (large sample sizes associated with high patchiness yielded spuriously high species richness), other estimators also failed.

MM, Chao 1 and ACE, abundance-based richness estimators, are highly sensitive to aggregated distribution of the species (Chazdon et al. 1998), as well as the incidence-based richness estimators (Butler & Chazdon 1998). Populations of Drosophilidae are patchily distributed (Tidon-Sklorz & Sene 1992) and, therefore, these estimators are not so effective for such assemblages. According to our data set, MM was the least erratic estimator (similarly to Chazdon et al. 1998) and it reached values closer to the final estimates with a smaller number of samples. However, in some cases the richness estimated by the MM method was lower than the number of species observed, what makes it a bad estimator for such data set (Palmer 1991). Chao 1 and ACE gave rise to the intermediate estimates (higher than those of MM and Bootstrap and lower than those of the remaining estimators).

We decided to suggest an interval of possible values for the species richness in the sampled sites and Piraí instead of electing a single value. This choice is related to the applicability restrictions of the estimators and the absence of asymptote in all the estimated species accumulation curves (Fig. 1). Thus, of the richness expected, 68.78 to 88.02% were observed in P1, 62.24 to 88.48% in P2, 65.40 to 87.47% in P3, and 69.35 to 88.81% in Piraí. Such values are satisfactory if we take into account the high richness of such fauna. However, the absence of asymptote in the estimated species accumulation curves indicates that these numbers would be altered if new samples were obtained.

Palmer (1991) analyzes the species richness of a vegetal community in the United States through six methods (including Jacknife 1, Jacknife 2 and Bootstrap). This author observed a high correlation between all of the richness estimators and the true richness. For this reason, he suggests that any method will suffice for comparing relative richness among sites. Thus, we suggest that any comparison with our richness estimates will be reliable if the same estimator is applied.

It is still important to highlight that there is always a finite number of species in a given area. However, for highly diverse areas, particularly with non-random spatial distributions, it might be necessary to sample the area completely in order to account fully for all of the species, especially the rare ones (Chazdon et al. 1998). Such situation seems to be the case of drosophilids in the studied area.

Previously, 118 species had been registered in the State of Santa Catarina (Gottschalk et al., manuscript in preparation). We collected seven species that had not been registered yet in this State and also in southern Brazil. One of these, *D. morelia*, had never been collected in South America (Table II). Hence, our collections improve the knowledge on the geographical distribution of such species, and increase the number of registered species in Santa Catarina from 118 to 125 (and to 305 in Brazil). The present study also evidences the high species richness and the large number of non-described species living in the Atlantic forests.

Acknowledgments. We thank MSc. Sabrina C. F. de Oliveira, Hermes J. Schmitz and Marco S. Gottschalk (UFRGS) for the discussions concerning the identification of several specimens; Dr. Francisca C. Val, Dr. Carlos Lamas (USP) and the Museu de Zoologia da Universidade de São Paulo for the permission to analyze the collection of Diptera; Dr. Milton Mendonça Jr. (UFRGS) for the help and suggestions concerning species richness estimation; Dr. Milton Mendonça Jr., Dr. Rosana Tidon (UnB), and the anonymous referees for their comments; and Aldo S. Döge for helping in the field work. This study is part of a Master's Dissertation (PPG Biologia Animal/Universidade Federal do Rio Grande do Sul-UFRGS) and it was partially supported by grants and fellowships from CAPES, CNPq and PROPESQ-UFRGS.

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Received 29/10/2007; accepted 07/07/2008