Journal of Medical Entomology, 2016, 1–13
doi: 10.1093/jme/tjw164
Research article

Sampling, Distribution, Dispersal

Assessment of Sampling Methods for Sarcosaprophagous Species and Other Guilds of Calyptratae (Diptera) in Temperate Forests of Southern South America

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Received 8 June 2016; Accepted 14 September 2016

Abstract

The aim of the present study was to compare three different collecting methods, namely, baited traps, active capture with hand net, and Malaise traps, to establish which method is more appropriate for sampling different Calyptratae guilds inhabiting temperate forests of South America. Specifically, it was analyzed which technique or combination of techniques is more adequate for obtaining sarcosaprophagous Calyptratae, which are of great interest from a veterinary and medical viewpoint. Taxa were classified into guilds according to their biology. Active capture was the technique that registered the highest diversity of guilds. When analyzing sarcosaprophagous species, it was observed that their percentage of captures, diversity, and abundance showed clear differences in guild composition between the trapping techniques studied. From these analyses it can be concluded that baited traps and active trapping are complementary methods for capturing sarcosaprophagous Calyptratae species. From the perspective of the biodiversity of this group, the combination of both methodologies allows obtaining a more complete inventory of sarcosaprophagous species of austral temperate forests of South America.

Key words: Sarcosaprophagous, guild, Diptera, Calyptratae, sampling method

In many studies on insect biodiversity, taxa are grouped in guilds according to their biological differences rather than according to a taxonomic point of view (Chapman and Sankey 1955, Mckinnerney 1978, Jirón and Cartín 1981, Braack 1987). Thus, guild structure analysis is a useful approach to examine the functional roles of species living in a particular community and a method to compare different communities. Typically, the guild concept has been usually applied to describe the spatial or temporal structure of ecological insect communities, taking into account the multiple ways in which species obtain food (Root 1967, Simberloff and Dayan 1991, Dilling et al. 2007, Wardhaugh et al. 2012). In contrast, other kinds of studies are targeted to explore diversity within a particular guild if such group of species causes high medical or sanitary impact on human activity (Arnaldos et al. 2001, Pohjoismäki et al. 2010, Battán Horenstein and Linhares 2011).

In any case, the application of adequate sampling methodology is crucial for the exploration of insect diversity at any given site. Insect catches are mostly based on many suitable methods and trap models to conduct biodiversity assessments. However, these samplings are subject to a number of inherent collection biases. Hence, the structure of the species assemblages obtained by these trapping methods is likely to vary according to the complex nature of the species (e.g., by their dispersal habits, life span, differential resource use, etc.) and the particularities of the trapping devices. Few studies have investigated differences in insect guild structure between different sampling techniques (Scheirs et al. 1997).

Calyptratae is one of the most species-rich and biologically diverse infraorder of Diptera (Yeates et al. 2007, Sujatha et al. 2010). This group includes many well-known groups of higher Diptera, as blow flies (Calliphoridae), flesh flies (Sarcophagidae), muscid flies (Muscidae), and tachinid flies (Tachinidae). Some families of Calyptratae are rather uniform in their biology, as it occurs with parasitic flies belonging to Tachinidae, whose species are exclusively parasitoids of other arthropods, especially other insects. Conversely, several other families are characterized by a wider range of biological traits, as it occurs with Anthomyiidae, Muscidae, and

Sarcophagidae (Marshall 2012). This proliferation of biological traits implies difficulties in collecting and inventorying Calyptratae, because it is unlikely to obtain representative samples with a single collecting technique (Brown et al. 2009). In the case of studies aimed to assess the biodiversity of Calyptratae, many decomposer species of Calliphoridae, Sarcophagidae, Fanniidae, and Anthomyiidae, whose larvae are scavengers or dung feeders, are usually included within the so-called sarcosaprophagous guild (Brown et al. 2009, Marshall 2012), and are more intensely sampled by their role as forensic indicators or pest status than other groups of calyptrate flies. Consequently, they are probably the groups of Calyptratae most studied from an ecological point of view. Indeed, a large proportion of diversity studies on Calyptratae are driven by biodiversity assessments derived from forensic succession experiments or sampling programs targeted to pest species.

The present study was performed in the context of explorations of the biodiversity of Calyptratae in the temperate forests of southern South America, and particularly focused on the characterization of flies whose biology suggest their medical, veterinary, or forensic importance (i.e., sarcosaprophagous flies). The temperate forests of southern South America have a highly endemic fauna of Diptera, which is still poorly known. In the case of Calyptratae, there are no studies on diversity assessment and structure of guilds associated with this ecoregion. Thus, the aim of this work was to assess the guild structures of Calyptratae obtained by using three different collecting methods, especially focusing on the diversity of sarcosaprophagous species. We also recorded the guild composition for each family of Calyptratae and compared the performance of the three techniques in relation to the collection of sarcosaprophagous Diptera. To this end, the proportional abundance, richness, and individual capture rate of sarcosaprophagous calyptrate flies were compared between the three sampling methods. Finally, a baseline inventory of the sarcosaprophagous Calyptratae found in the temperate forests of southern South America was obtained.

Materials and Methods

Study Area

The Valdivian temperate forests of austral South America form an ecoregion that covers a narrow 100–250-km-wide strip along the South American Pacific coast between 37 and 48° S (Cabrera and Willink 1973, Olson et al. 2001; Fig. 1a). The mean temperature varies between 21°C and 13°C in the northern and southern ends of the ecoregion, respectively, whereas annual precipitation ranges between 1,000 mm and 6,000 mm per year, concentrated in winter, and decreasing in the eastern slopes of Andes.

Sampling sites ($n = 15$) were distributed in two areas: Lanín National Park (LNP) and Lago Puelo National Park (LPNP), which are located in Neuquén and Chubut provinces, respectively, Argentina (Fig 1b-c). Samplings were performed during the summer, which represents a dry season, and in two years in each park. In LNP, sampling was performed in February 2011 and January 2013, whereas in LPNP, sampling was performed in January 2011 and January 2012. All sites were selected as representative of typical forms of the temperate forest.

The samples were taken at different points of the LNP (Fig. 1b). Sites located in the northern sector of the park were: 1) Ñorquinco (−39.15, −71.25); 2) Ruca Choroi (−39.2166, −71.1666); and 3) Quillen (−39.3613, −71.2188). Whereas the sites located in the southern sector were: 4) Seccional Bandurrias (−40.1448,

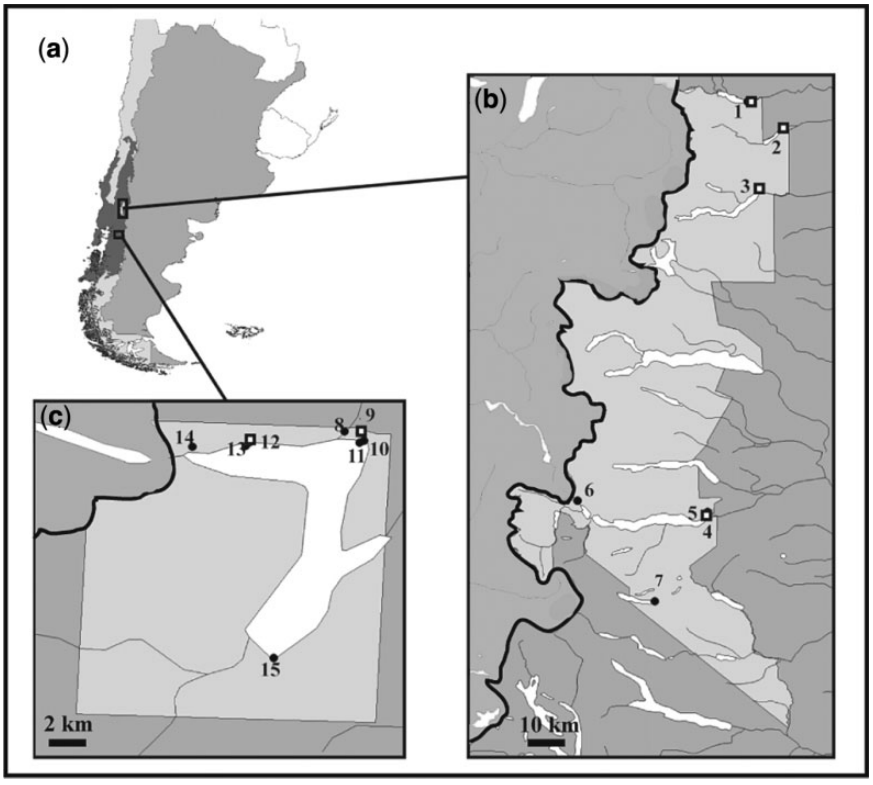

Fig. 1. Map of the study sites. (**a**) Map of Valdivian temperate forests of austral South America (in darker gray), (**b**) Map of Lanín National Park (LNP). c) Map of National Park Lago Puelo (LPNP). Sampling sites (black circle); sampling sites where the three techniques (baited trap, active capture, and Malaise trap) were applied (square white).

−71.3471); 5) Mirador Bandurrias (−40.1617, −71.2659); 6) Hua Hum (−40.1534, −71.3539); and 7) Laguna Pudú Pudú (−40.3620, −71.4749). Furthermore, sampling points in the LPNP (Fig. 1c) were placed in the following sites: 8) Río Azul 1 (−42.0916, −71.6155); 9) Río Azul 2(−42.0908, −71.6247); 10) Pitranto Grande (−42.0963, −71.6129); 11) La Playita (−42.0974, −71.6155); 12) Gendarmería 1 (−42.0973, −71.6821); 13) Gendarmería 2 (−42.0994, −71.6845); 14) Los Hitos (−42.1, −71.7166); and 15) Rio Turbio (−42.2280, −71.6675).

Sampling

We perform comparisons between baited traps, a technique frequently used to obtain sarcosaprophagous flies, and two other different techniques: active capture with hand net and Malaise traps. Baited traps consisted of a modification of the bottle trap used by Hwang and Turner (2005). These traps have at their base a plastic jar measuring approximately 150 mm in diameter and 200 mm in height. They have four lateral openings and a funnel in their upper part, manufactured with a plastic bottle, which allows the entry of dipterous insects but blocks their way out, through another bottle placed above. In the interior of the plastic jar, there is a container covered by a piece of Lycra, where the bait is placed. The bait used in the traps was bone meal (putrescine), which is a foul-smelling organic chemical compound produced by the breakdown of amino acids in living and dead organisms. These baits were placed in all selected sites of both national parks at 10:00 am and extracted at approximately 04:00 pm. Four to nine traps were simultaneously placed within each site at shaded positions to avoid thermal stress for the captured flies (Table 1).

For the active capture method, all specimens encountered were caught with an entomological net while foraging on flowers or vegetation, resting on soil or stones, or in flight. These captures took place in areas adjacent to those sites where the baited traps were placed (15 sampling localities). These captures were done for 3 h by three researchers at each site of the areas studied, totalizing six active capture units per site (Table 1).

The Malaise traps used during this study belonged to the Townes' model (Townes 1972). Inside the collecting jar of the trap, a small container with ethyl acetate was used to kill the specimens, previously covering it with Lycra to keep the material dry. The collecting jar was examined daily. The traps were placed perpendicularly to the edge of forests when possible, depending on the conditions of each site, and worked a variable amount of time at each site. Six sites shared the three sampling methods. The Malaise traps worked a variable amount of time at each of these sites depending on the availability, accessibility, and safety conditions of the site. Whenever the sites were under attendance of the personnel of the parks, the Malaise traps were left for longer periods (Table 1). In all cases, these traps operate at the same time than the baited traps and the active captures at least for 6 h. For comparative purposes, overall fly counts for each sampling technique were standardized by means of the relative abundance obtained for each guild. In addition, some comparisons of richness or capture rate were standardized as number of flies captured per hour per sampling device (or collector) to obtain comparative values independently of cumulative time of sampling or number of trap units.

Identification, Preservation, and Classification of Specimens

Dry insects are very delicate and care must be taken to avoid specimens from losing legs, heads, or antennae during transport. Consequently, dried flies were mounted with pins or minuten after each capture date. This first sorting and pinning of samples in the field were done with the use of a Leica ES2 stereomicroscope. Specimens were identified at the species level or, when not possible, at the lowest taxon possible, using dichotomous keys and specific taxonomic revisions and descriptions available for each group (Malloch 1934, Hall 1937, de Carvalho 2002, Mulieri et al. 2014, Mulieri et al. 2015b). All specimens were labeled and deposited in the entomological collection of Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN), Buenos Aires, Argentina.

Specimens were grouped according to trophic guild. For this purpose, we adopted the general concept of "structural guild," which defines a group of species that use the same resource, although not necessarily in the same way or for the same purpose (Szaro 1986). This criterion was adopted because the designation of adult flies into guilds is problematic and limits between guilds are frequently blurred (Kitching et al. 2005, Hanski 1987a). Furthermore, this basic concept allowed us to include each species into a single guild according to the available information and to reflect the kind of foraging substrate exploited. Indeed, the foraging substrate refers to the place where organisms obtain their food, specifically to the substrate

Table 1. Baited traps, active captures, and Malaise traps distributed in 15 localities in LNP and LPNP

Locations	Date	Baited trap	Active capture	Malaise trap (hours per Malaise)
LNP				
Hua Hum	Feb-2011	6	6	–
Mirador Bandurrias	Feb-2011	6	6	–
Seccional Bandurria	Feb-2011	6	6	2 (144h)
Laguna Pudú Pudú	Feb-2011	6	6	–
Ñorquinco	Jan-2013	9	6	2 (6h)
Ruca Choroi	Jan-2013	9	6	2 (6h)
Quillen	Jan-2013	9	6	2 (6h)
LPNP				
Pitranto Grande	Jan-2011	6	6	–
Río Turbio	Jan-2011	6	6	–
Los Hitos	Jan-2011	6	6	–
Río Azul 1	Jan-2011	6	6	2 (6h)
Gendarmería 1	Jan-2011	4	4	2 (120h)
La Playita	Jan-2012	4	4	–
Gendarmería 2	Jan-2012	4	4	–
Río Azul 2	Jan-2012	4	4	–

where their larval instars can develop or the interaction of the adults with a given limiting resource (Kitching et al. 2005).

Hence, to obtain a single guild assignation for each taxon, the following stepwise criteria were adopted: 1) Each species was assigned to a single guild on the basis of the information related to the larval breeding substrate. 2) In the absence of such information, the second option was to extrapolate the available data on breeding substrates recorded from closely related taxa. Such extrapolation was extracted from co-generic species or supraspecific taxa, accordingly. 3) Finally, if there was no information available on breeding substrate used by the immature specimens, existing records on adult feeding behavior were taken into account (or extrapolated from related taxa). For each taxon, we recorded the criteria used.

Taking into consideration the above criteria, the following guilds of Calyptratae were considered:

Coprophagous: Species that feed on feces or dung (Hanski 1987b).

Necrophagous: Species organisms that feed on carrion (Hanski 1987b).

Generalists saprophagous: We placed here those flies species that feed indistinctly on feces or carrion. Specifically, all species that behave indistinctly as coprophagous or necrophagous. Those species whose habits are ambiguously recorded in the literature were included here.

Detritivorous: This term was applied only to the species associated with decaying organic matter that is not primarily composed of animal protein (as feces or carrion). These species are usually associated to debris in humid environments (i.e., moss, mud), and in certain circumstances with some degree of phytophagy. In many cases, it includes species with predaceous adults as seen with the Coenosiinae muscids.

Kleptoparasites: Species that develop at the expense of another host organism, through the misappropriation of its food, ending up killing the host either directly or indirectly as a result (Eggleton and Belshaw 1992).

Parasitoids: Species that develop over or inside a host, from which they extract nutrients, causing them to die either directly or indirectly (Kuris 1974).

These guilds were clustered into the following hierarchical ordination (Table 2): parasites and decomposers. The former included kleptoparasites and parasitoids, whereas the latter included all the organisms feeding on decomposing organic matter. In this group, two main subgroups of guilds clearly emerged on the basis of the type of foraging substrate and their intrinsic dynamics: sarcosaprophagous species, which included guilds associated with patchily distributed and ephemeral resources with high content of animal protein (feces, carcasses), and detritivores, whose breeding substrate had lower contents of animal protein, was not ephemeral, and did not occur as patchy microhabitats.

Table 2. Classification scheme of the structural guilds for Calyptratae species

Decomposers		
	Sarcosaprophagous	*Coprophagous*
		Necrophagous
		Generalist saprophagous
	Detritivorous	*Detritivorous*
Parasites		
		Kleptoparasites
		Parasitoids

Data Analysis

Diversity and Abundance

The performance of each method was evaluated by analyzing the proportional number of captures of each guild. To estimate and compare the diversity of the different guilds derived from each type of sampling, the Shannon (H) index was calculated (Magurran 2004). This index and confidence intervals (CI) were obtained using Infostat software (Di Rienzo et al. 2013). The same analysis was applied to analyze the species diversity of sarcosaprophagous flies. Also, differences in proportional occurrence of sarcosaprophagous flies between sampling techniques were assessed by means of the test for independent proportions (Fleiss 1981).

In addition, we generated sample-based rarefaction curves to compare the richness of sarcosaprophagous species obtained through each type of sampling method. Rarefaction curves allow standardizing samples of different size to establish comparisons between them, through repeated random resampling of a group of N collected samples (Gotelli and Colwell 2001). Rarefaction curves were developed with the program PAST (Hammer et al. 2001).

Rank abundance plots of taxa were compared to examine relative abundance patterns between sampling methods to show the extent of variation between them (Sackmann 2006). In such analysis, baited traps were arranged as reference with regard to the other methods.

Capture Rate. The capture rate was compared between collecting methods. For this analysis, only six sites, where the three sampling techniques were included (Fig. 1b, c), were taken into account. To perform this analysis, the number of specimens collected per hour per sample unit for each sampling method was calculated. A nonparametric Friedman test for dependent samples n (Zar 1996) was applied to analyze whether there were differences in catch rates between the different methods.

Results

In total, 5,550 specimens were collected during the study. In LNP, 2,193 specimens were captured, with the highest number obtained through baited traps (1,326 individuals; 63.5%), followed by active capture (658 individuals; 30.0%) and Malaise traps (209 individuals; 9.5%). In LPNP, 3,357 specimens were captured, with 2,221 specimens (66.2%) captured with baited traps, 717 individuals (21.35%) obtained through active capture, and 419 individuals (15.31%) captured with Malaise traps. The capture percentage in both parks was similar.

A baseline inventory of 37 species of sarcosaprophagous flies was recorded (17 necrophagous, 4 coprophagous, and 16 generalist saprophagous flies), of which only 11 species had direct observations and records of their exploited breeding substrates (Table 3).

Guild Composition

The percentage of guilds varied across the sampling methods. Baited traps exhibited the highest capture percentage of necrophagous, coprophagous, and other generalist saprophagous species. Indeed, this capture method allowed obtaining sarcosaprophagous species almost exclusively (98-99%). In comparison, the active capture obtained 50% of sarcosaprophagous species, followed by detritivorous and parasitoid species. Finally, Malaise traps allowed capturing a higher proportion of detritivorous, followed by parasitoids and generalist saprophagous species, respectively. These trends showed a roughly similar pattern in both national parks (Table 4).

Table 3. Designation of each taxon into different guilds

Family	Taxa	Structural guild	References	Criteria
Anthomyiidae	sp. 1	Generalist saprophagous	Michelsen 2010	2
	sp. 2	Generalist saprophagous	Michelsen 2010	2
	sp. 3	Generalist saprophagous	Michelsen 2010	2
	sp. 4	Generalist saprophagous	Michelsen 2010	2
Calliphoridae	*Calliphora vicina* Robineau-Desvoidy, 1830	Necrophagous	Camacho 2005	1
	Compsomyiops fulvicrura (Robineau-Desvoidy, 1830)	Necrophagous	Trigo 2006	1
	Lucilia sericata (Meigen, 1826)	Necrophagous	Pinilla et al. 2010	1
	Sarconesia chlorogaster (Wiedemann, 1830)	Necrophagous	Vairo et al. 2015	1
	Sarconesiopsis magellanica (Le Guillou, 1842)	Necrophagous	Pinilla et al. 2013	1
Fanniidae	*Fannia* sp.1	Generalist saprophagous	Savage and Vockeroth 2010	2
	Fannia sp. 2	Generalist saprophagous	Savage and Vockeroth 2010	2
	Fannia sp. 3	Generalist saprophagous	Savage and Vockeroth 2010	2
	Fannia sp. 4	Generalist saprophagous	Savage and Vockeroth 2010	2
	Fannia sp. 5	Generalist saprophagous	Savage and Vockeroth 2010	2
Muscidae	*Apsil spp*	Detritivorous???	No information	
	Arthurella nudiseta Albuquerque, 1954	Generalist saprophagous	Lopes 1985; Patitucci et al. 2011	2,3
	Coenosia spp	Detritivorous	Skidmore 1985	2
	Helina spp	Detritivorous	Skidmore 1985	2
	Hydrotaea acuta Stein, 1898	Necrophagous	Skidmore 1985	2
	Hydrotaea cyaneiventris Macquart, 1851	Necrophagous	Skidmore 1985	2
	Lispe sp.	Detritivorous	Savage and Vockeroth 2010	2
	Lispoides spp	Detritivorous/Predator	Skidmore 1985	2
	Muscina stabulans (Fallén, 1817)	Generalist saprophagous	Skidmore 1985	1
	Myospila cyanea (Macquart, 1843)	Generalist saprophagous	Savage and Vockeroth 2010	2
	Ophyra aenescens (Wiedemann, 1830)	Necrophagous	D'Almeida et al. 1999	1
	Ophyra sp.	Necrophagous	Skidmore 1985	2
	Palpibracus spp	Generalist saprophagous???	Figueroa-Roa and Linhares 2004	3
	Psilochaeta apicalis (Malloch, 1934)	Generalist saprophagous???	Figueroa-Roa and Linhares 2004; Patitucci et al. 2013	3
	Psilochaeta chalybea (Wiedemann, 1830)	Generalist saprophagous	Figueroa-Roa and Linhares 2004; Patitucci et al. 2013	3
	Reynoldsia spp	Detritivorous	No information	
	Schoenomyza spp	Detritivorous	Skidmore 1985	2
	Schoenomyzina spp	Detritivorous?? ?	No information	
	Spathiphermyia spp	Detritivorous	Skidmore 1985	2
	Syllimnophora spp	Detritivorous	Skidmore 1985	2
Sarcophagidae	*Microcerella chilensis* (Hall, 1937)	Necrophagous	De Arriba and Costamanga 2006; Moretti et al. 2009; Moura 2004; Mulieri et al. 2012	2
	Microcerella coniceti Mariluis, 2006	Necrophagous	De Arriba and Costamanga 2006 Moretti et al. 2009; Moura 2004; Mulieri et al. 2012	2
	Microcerella edwardsi (Hall, 1937)	Necrophagous	De Arriba & Costamanga 2006; Moretti et al. 2009; Moura 2004; Mulieri et al. 2012	2
	Microcerella spinosa (Hall, 1937)	Necrophagous	De Arriba and Costamanga 2006; Moretti et al. 2009; Moura 2004; Mulieri et al. 2012	2
	Microcerella mallochi (Hall, 1937)	Necrophagous	De Arriba and Costamanga 2006; Moretti et al. 2009; Moura 2004; Mulieri et al. 2012	2
	Microcerella sp.	Necrophagous	De Arriba and Costamanga 2006; Moretti et al. 2009; Moura 2004; Mulieri et al. 2012	2

(continued)

Table 3. Continued

Family	Taxa	Structural guild	References	Criteria
	Microcerella spinigena (Rondani, 1863)	Necrophagous	De Arriba and Costamanga 2006; Moretti et al. 2009, Moura 2004; Mulieri et al. 2012.	2
	Microcerella rusca (Hall, 1937)	Necrophagous	De Arriba & Costamanga 2006; Moretti et al. 2009; Moura 2004; Mulieri et al. 2012	2
	Opsidia intonsa Aldrich, 1928	Kleptoparasites	Pape 1989	2
	Oxysarcodexia varia (Walker, 1836)	Coprophagous	Hernandez 1989	1
	Oxysarcodexya bikini Dodge, 1966	Coprophagous	Hernandez 1989	2
	Ravinia aureopyga (Hall, 1928	Coprophagous	Blanchard 1939	1
	Sarcophaga argyrostoma (Robineau-Desvoidy, 1830)	Coprophagous	Grassberger and Reiter 2002	1
	Tricharaea sp.	Generalist saprophagous	Lopes 1973	2
Tachinidae		Parasitoids	Wood and Zumbado 2010	2

Criteria of assignment: (1) direct information on larval substrate, (2) information on larval substrate extrapolated from nearest taxa, (3) information from the adult of the same taxa.

Table 4. Abundance and percentage of flies by structural guilds and sampling method in LNP and LPNP

Guild	Baited trap				Active capture				Malaise trap			
	LNP		LPNP		LNP		LPNP		LNP		LPNP	
	N	%	N	%	N	%	N	%	N	%	N	%
Necrophagous	497	37.5	951	42.8	125	19.5	134	18.7	4	2.1	5	1.2
Coprophagous	171	12.9	823	37.1	32	5.8	131	18.3	1	0.5	2	0.5
Generalist saprophagous	627	47.2	421	19.0	147	23.4	96	13.4	36	19.0	87	20.8
Detritivorous	31	2.3	26	1.2	136	21.4	262	36.6	74	39.2	246	58.7
Kleptoparasites	0	–	0	–	28	2.0	32	4.4	3	1.6	4	1.0
Parasitoids	0	–	0	–	173	27.9	60	8.3	71	37.6	75	17.9

The diversity index (Fig. 2) in both national parks indicates that active capture was the technique through which the highest guild diversity was recorded. This was followed by Malaise traps, although the confidence intervals associated with the index suggest no significant differences in guild diversity between Malaise traps and baited traps.

The sample of sarcosaprophagous species was dependent on the sampling methodology, as is shown by the significant differences in proportional abundance of this group of guilds between the three sampling methods (LNP $\chi^2 = 1035.73$, $df = 2$, $P < 0.05$; LPNP $\chi^2 = 1789.44$, $df = 2$, $P < 0.05$; Fig. 3).

The families of Calyptratae recorded during the study were Muscidae, Fanniidae, Anthomyiidae, Sarcophagidae, Tachinidae, and Calliphoridae. Analysis of the percentage of guilds within each family showed a higher diversity of guilds in Sarcophagidae and Muscidae. The family Tachinidae was totally composed of parasitoid species, the Anthomyiidae and Fanniidae were composed of sarcosaprophagous species, and the Calliphoridae was composed of necrophagous species. The family Sarcophagidae was represented by coprophagous (56.8% LNP and 79.6% LPNP), necrophagous (39.3% LNP and 16.3% LPNP), and kleptoparasite species in a lower proportion (2.9% LNP and 3.9% LPNP). The Muscidae, on the other hand, was mainly represented by detritivorous (55.9% LNP and 51.1% LPNP) and necrophagous (33.9% LNP and 32.9% LPNP) species, with a lower proportion of sarcosaprophagous species (10.2% LNP and 16% LPNP; Fig. 4 a-b). The percentage represented by each guild in the different families of Calyptratae was similar, although the abundance captured in the two parks was quite different (Fig. 4 c-d).

The samples obtained through different sampling methods showed that Muscidae presented higher capture percentage with Malaise traps (Table. 5). In LNP, Anthomyiidae showed similar percentages with the three methods, whereas in LPNP, its percentage was higher with Malaise traps. Sarcophagidae exhibited notably lower capture proportion with Malaise traps than with the other methods. Tachinidae did not present any record with baited traps, with similar percentages with active capture and Malaise traps. In contrast, the family Calliphoridae showed higher capture percentage with baited traps and active capture (Table 5).

Diversity of Sarcosaprophagous Calyptratae

The diversity index calculated for sarcosaprophagous species indicates that in LNP, baited traps exhibited higher diversity, in contrast to the situation in LPNP, where the method with higher values was the active capture (Fig. 5).

The rarefaction analysis based on samples revealed differences in sarcosaprophagous species richness between the three sampling methods. Rarefaction curves for baited traps accumulated species more rapidly, followed by active capture and Malaise traps, respectively, although no rarefaction curve reached the asymptote (which is a sign of inventory completeness). Both areas revealed the same results (Fig. 6).

Rank-abundance diagrams show that species abundance patterns were markedly different between sampling methods but suggest

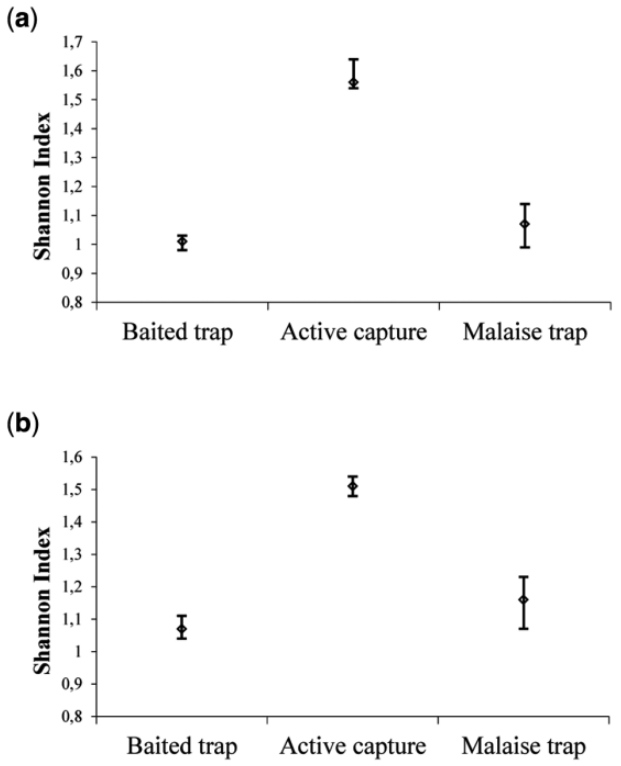

Fig. 2. Diversity of structural guilds obtained in each sampling technique in (**a**) Lanín National Park and (**b**) Lago Puelo National Park.

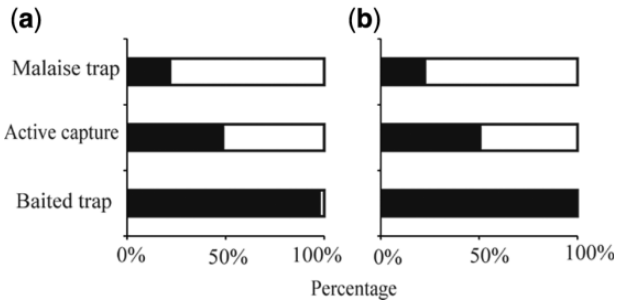

Fig. 3. Percentage of sarcosaprophagous (necrophagous + coprophagous + saprophagous generalist) dipterous species captured by the three analyzed sampling methods in (**a**) Lanín National Park and (**b**) Lago Puelo National Park. Sarcosaprophagous (black); other guilds (Detritivorous+ Parasites) (white).

certain complementarity between them (Fig. 7). Baited traps recorded a higher number of species than the other two methods. However, certain species that were either not collected or simply represented by few specimens on baited traps possessed a relatively higher rank for the other collecting methods (Fig. 7).

Capture Rate

The rate of Calyptratae specimens obtained by time unit with the different methods showed no significant differences (Friedman ANOVA $\chi^2 = 4.33$; $N = 6$; df = 2; $P > 0.05$; Fig. 8a). However, when exclusively evaluating the capture rate of sarcosaprophagous specimens, the results obtained showed significant differences between the sampling methods (Friedman ANOVA $\chi^2 = 12.00$; $N = 6$; df = 2; $P < 0.05$; Fig. 8b). In the latter case, baited traps showed the highest capture rate.

Discussion

Diverse trapping techniques are frequently used to assess biodiversity or monitor a given group of flies. This study compared the effectiveness of three of the methods used to collect flies: baited traps, active capture with entomological nets, and Malaise traps. We compared the composition of samples to describe the suitability of each technique to determine the diversity of sarcosaprophagous flies in the temperate forests of southern South America. Our study showed clear differences in guild composition between the trapping techniques studied.

These three sampling techniques involve well-differentiated capture mechanisms that are exposed to different factors that affect their efficiency, or imply particular capture biases. The baited trap is a device that concentrates the majority of flies within its influence by means of use of an effective attractant (Muirhead-Thomson 1968). Baited traps are mainly influenced by the selection of bait, which is typically rotten fruits, feces, or rotten animal tissues (D'Almeida, 1986, 1989, 1993, 1994), but may also be influenced by the age or stage of drying of the bait used. As sarcosaprophagous species are mostly attracted to bait of animal origin, the selection of uniform and easily replicated bait was crucial to our study. However, it is accepted that different baits attract different species. Comparative studies between different baits have demonstrated that Sarcophagidae are mostly captured by feces baits (D'Almeida 1986, 1994, Mendes and Linhares 1993, Mulieri et al. 2011, 2015a). This behavior is typically recorded in the genera *Ravinia* and *Oxysarcodexia*, which include many species with life habits related to feces as a substrate in which their larvae may develop. In contrast, species of Calliphoridae usually show preference for carrion baits (Linhares 1981, Baumgartner and Greenberg 1984, Mulieri et al. 2006) and are the most ubiquitous necrophagous flies. Our observations suggest that putrescine baits seem to be highly effective for the capture and, hence, to obtain records of coprophagous and necrophagous species. However, to reliably verify whether putrescine provides intermediate capture conditions between feces and carrion, comparative tests between these three substrates should be performed in future works.

During this study, the baited traps did not collect any tachinid fly. This result may be expected, as the usual feeding habits of the adult tachinids on flowers or honey dew may be better reflected by catches performed with the active capture method. However, there are some reports in the literature about the presence and collection of small numbers of tachinids on carcasses or carrion-baited traps (Gómez-Gómez et al. 2010).

In relation to the active capture method, it is fundamentally a collector-dependent method. This procedure is not easily replicated and may involve high effort costs to the persons that perform the catches. Its efficiency may be highly variable according to different characteristics of the person involved, such as their physical condition, ability to capture flies, acquired experience, or particular interest toward certain specific taxa. Another important factor is the characteristics of the terrain, which influences the facility to circulate and perform the captures. Diptera features, such as their size or behavior, can also influence the effectiveness of this method. Thus, highly mobile flies are usually more difficult to collect if the person has little experience, while visualization and capture can be favored in the case of species that rest in exposed rocks and branches, or

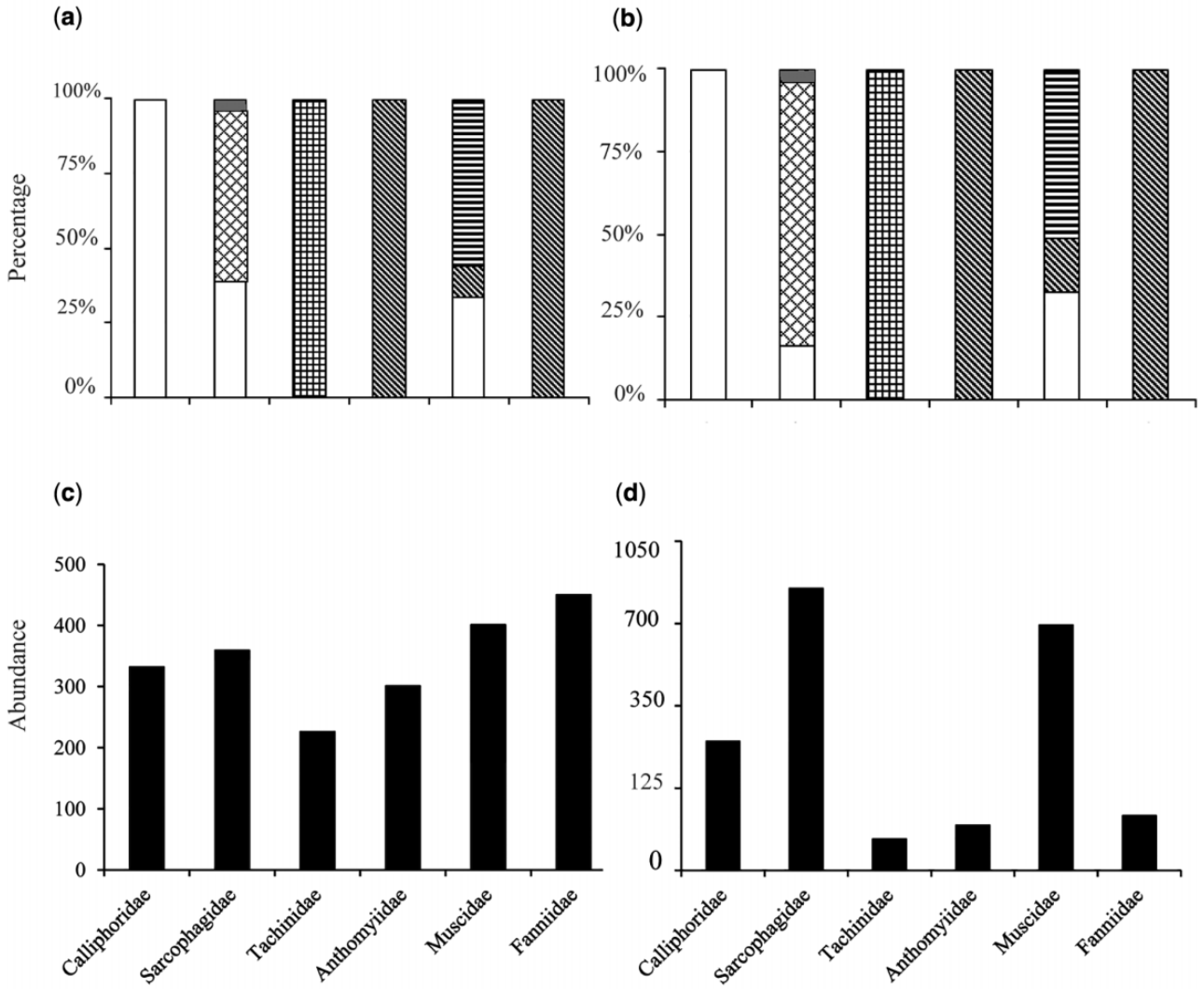

Fig. 4. a-b Guild percentage within each family of Calyptratae species. (**a**) Lanín National Park. (**b**) Lago Puelo National Park. Necrophagous (white), kleptoparasites (gray), coprophagous (oblique square); parasitoids (square), generalist saprophagous (oblique line), and detritivorous (horizontal line). **c-d**. Abundance of Calyptratae families captured within each family. (**c**) Lanín National Park; (**d**) Lago Puelo National Park.

Table 5. Abundance and percentage of Calyptratae families obtained with the three sampling methods in LNP and LPNP

Families	Baited trap				Active capture				Malaise trap			
	LNP		LPNP		LNP		LPNP		LNP		LPNP	
	N	%	N	%	N	%	N	%	N	%	N	%
Anthomyiidae	190	14.3	75	3.4	79	12.0	37	5.2	32	15.3	79	18.9
Calliphoridae	301	22.7	473	21.3	31	4.7	75	10.5	0	–	1	0.2
Fanniidae	409	30.8	214	9.6	42	6.4	27	3.8	0	–	1	0.2
Muscidae	190	14.3	499	22.5	234	35.6	291	40.6	100	47.8	253	60.4
Sarcophagidae	236	17.8	960	43.2	117	17.8	229	31.9	6	2.9	10	2.4
Tachinidae	0	–	0	–	155	23.6	58	8.1	71	34.0	75	17.9

perform territorial displays. On the other hand, any small-sized dipterous species with inconspicuous behavior might be subsampled by this method.

These intrinsic features of capture methods contribute to obtaining contrasting information about patterns of abundance and spatial distribution of the species studied. In fact, due to their capture specificity, baited traps can be more effective to estimate both the taxonomic composition of sarcosaprophagous assemblages and species abundances. Nevertheless, these characteristics do not exclude the existence of differential attraction biases within species (Mulieri

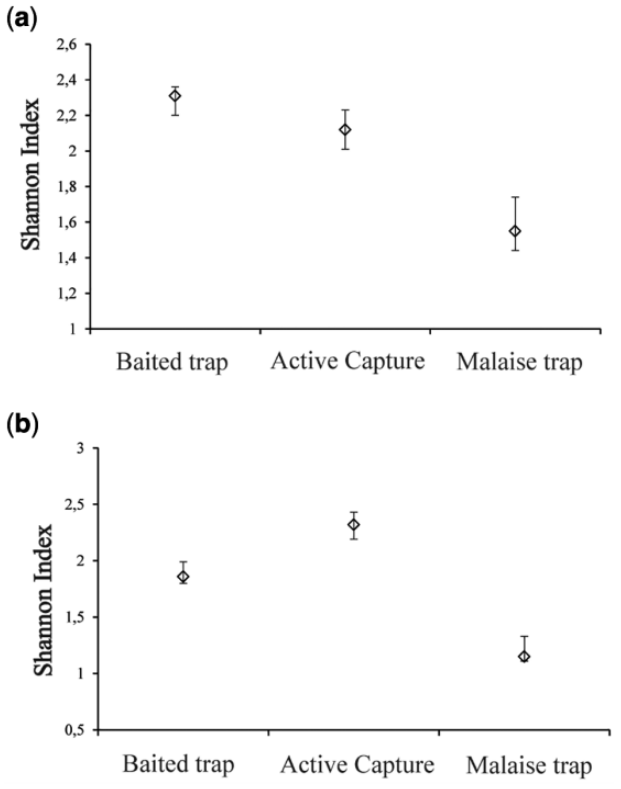

Fig. 5. Diversity of sarcosaprophagous Calyptratae captured with the three methods in (**a**) Lanín National Park and (**b**) Lago Puelo National Park.

et al. 2015a). In contrast, active capture might be a potential useful tool to analyze the use of habitat types or natural resting places of different species, as it does not imply the concentration of specimens from surrounding areas due to the bait effect and does not affect species behavior derived from the attractants. In addition, the sex ratio of the species may be affected by trapping methodology. However, this analysis is beyond the scope of this study.

Finally, the functioning of Malaise traps is highly dependent on their location. It is generally considered that placing the trap perpendicularly to the edge of the forest is most favorable for collection of flies (Brown et al. 2009). In this study, in some sample sites, the vegetation physiognomy did not allow for an adequate positioning of the traps. On the other hand, as Malaise traps perform random interception, they are considered a very effective method to capture rare or small-sized species (Brown 2005). For instance, a high proportion of detritivores was captured with this method along the study, many of them being small muscid flies belonging to Coenosiinae, which usually have been overlooked by collectors during active capture.

To our knowledge, few studies have assessed the guild composition of Diptera, particularly of Calyptratae, in sampling programs with multiple techniques (Kitching et al. 2005). This is the first study describing the relative importance of the different guilds that integrate this infraorder, conducted in temperate austral forests of South America. In South America, most studies have focused on the faunistic composition of Calyptratae collected with different types of bait (D'Almeida 1986, 1989, 1993; Mulieri et al. 2006). Furthermore, the biodiversity of Calyptratae explored with Malaise traps or active capture has been little explored (Brown 2005). In the temperate

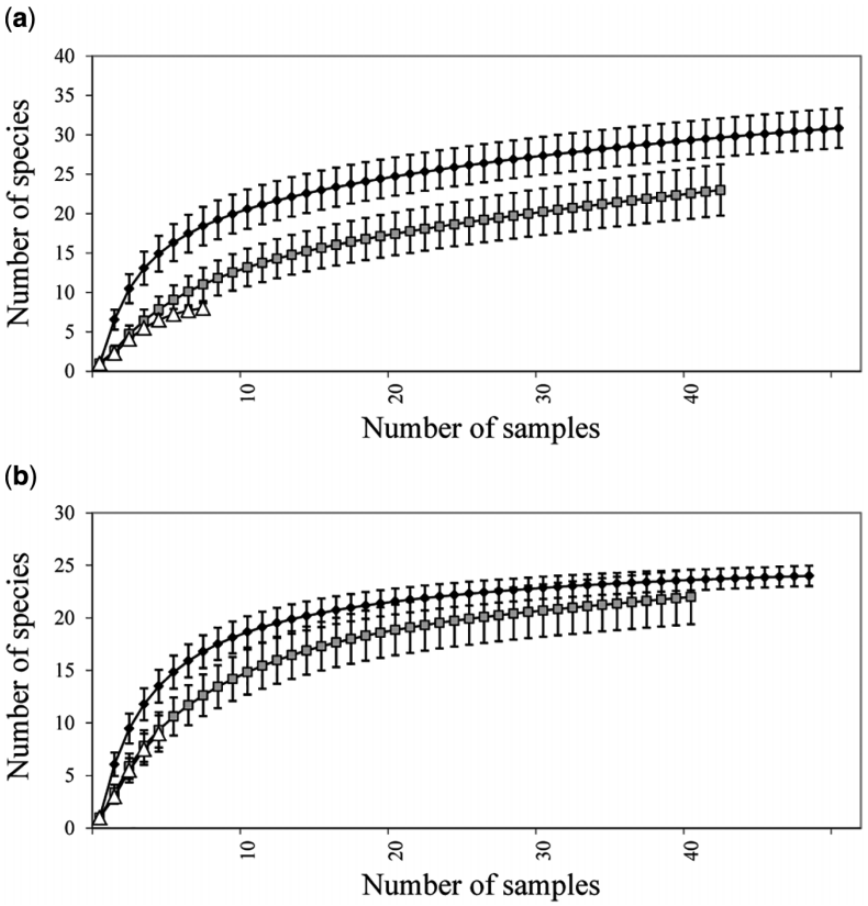

Fig. 6. Rarefaction curves of Calyptratae sarcosaprophagous based on samples derived from the three sampling methods: baited traps (black circle), active capture (gray square), and Malaise traps (white triangle). (**a**) Lanín National Park, (**b**) Lago Puelo National Park.

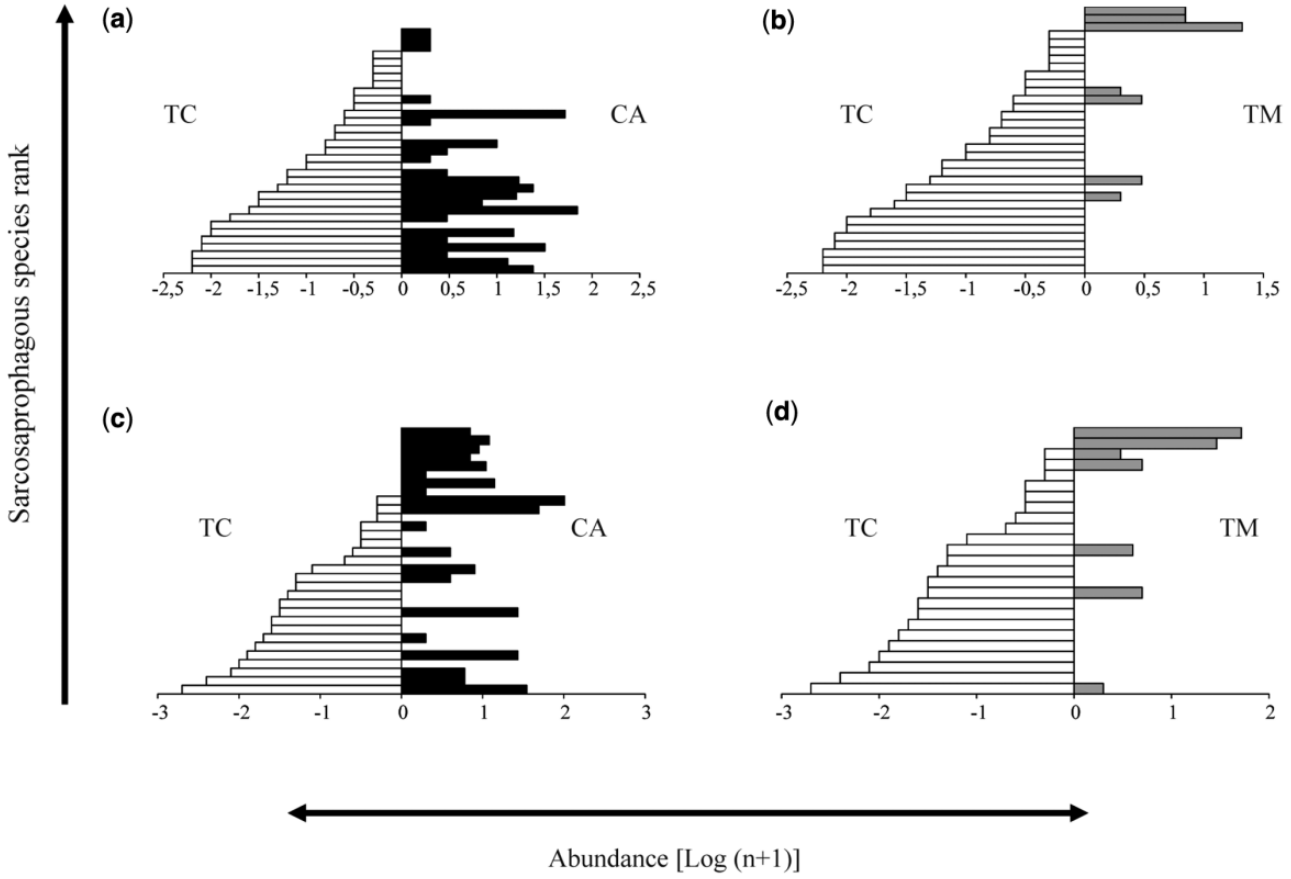

Fig. 7. Comparison of the three sampling techniques used in this study (a and b) in Lanín National Park and (c and d) Lago Puelo National Park. The right side of the figures shows the relative abundance of Calyptratae collected with baited traps (white), ordered from highest to lowest. In the left side, the relative abundance of the same species collected with active capture (black) (a and c) and Malaise traps (gray) (b and d) is shown. Baited trap (white), active captures (black), and Malaise trap (gray). Baited trap (BT), active capture (AC), and malaise trap (MT).

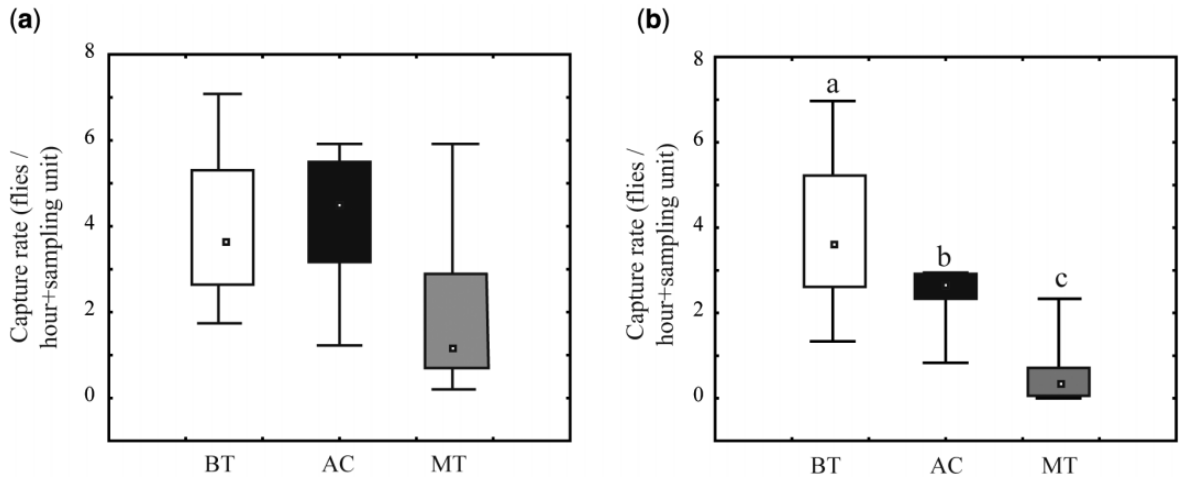

Fig. 8. Capture rate obtained with the three sampling methods. (a) Calyptratae, (b) sarcosaprophagous Calyptratae. Baited trap (BT), active capture (AC), and Malaise trap (MT).

austral forests of South America, previous works have focused on Calliphoridae species exclusively (Mariluis and Schnack 1996, Mariluis et al. 1999).

Considering the lack of a unified classification for ecological guilds (González-Salazar et al. 2014), the available information on life cycles and biological traits of Neotropical Calyptratae was reviewed to provide a classification scheme for Calyptratae of temperate forests of southern South America. Specifically, our results provide a baseline inventory of sarcosaprophagous species, with potential medical or forensic impact, inhabiting the temperate forests

of southern South America. Several species of this group still lack direct observations on their breeding substrates (e.g. only one-third of species here recognized as necrophagous have direct records on exploited substrates). However, the results obtained may offer valuable insights into the patterns of resource use of these taxa, and will allow hypothesizing on their specific biological traits. In such cases, the assignation into the sarcosaprophagous guild, extrapolated from the nearest taxa, should be specifically tested.

Several studies have used the resource exploited by the species as the sole criterion to define guilds, regardless of the way they exploit the resource (Cagnolo et al. 2002, Feeley 2003, Aragón et al. 2009). A problem with using such coarse categories is that species overlap on the resource used. In contrast with other orders such as Lepidoptera or Hymenoptera, with more uniform life strategies, the order Diptera exhibits a wide range of life strategies, and is thus adequate for the classification of different guilds as a function of their life cycles (Kitching et al. 2005). However, guild classification based exclusively on adults is problematic, because along their life cycle, a given species can resort to many different resources. In fact, many species are generalist and can thus belong to more than one guild. In Sarcophagidae, Muscidae, and Calliphoridae, many of the adults search for sugar and protein in their adult stage, either due to trophic or reproductive requirements (Roberts and Kitching 1974). In this case, protein is the limiting resource, as sugar sources are highly available. Thus, their classification as sarcosaprophagous is related to the importance of the resource for completing reproduction, even when, during their adult stage, species can use other trophic resources. These features do not make guild classification as something restricted to immature stages. Indeed, the designation of larvae as sarcosaprophagous carries the problem that it is difficult to differentiate true saprophagous species from those that feed on microorganisms associated with debris, or those that are predators of other larvae that colonize organic matter substrates (Kitching et al. 2005, Mulieri et al. 2015a).

When specimens are assigned to guilds, an important point is the fact that biologically complex and largely unstudied families are the ones that may exhibit problems in their assignation. In this work, specimens of Anthomyiidae were classified at family level and were considered saprophagous as a whole. It is known that species of this family present other life strategies (e.g., kleptoparasitic species; Brown et al. 2009). Thus, it is likely that at least a given fraction of Anthomyiidae in the study area belong to guilds other than the sarcosaprophagous one. However, confident guild assignations are not needed on all the families recorded to be able to carry out a robust statistical analysis (Kitching et al. 2005).

To acquire reliable and complementary information on complex communities, it is necessary to perform a combination of sampling techniques (Martikainen and Kouki 2003, Ozanne 2005). In this study, the captures of species that exploit ephemeral patches of animal protein were specifically analyzed, and this included a wide range of coprophagous, necrophagous, and generalist saprophagous species. Both baited traps and active capture were the most efficient methods, whose samples recorded higher richness than Malaise traps. However, baited traps sampled higher species richness, but with low equitability (i.e., capturing only a few species with high abundance). On the other hand, the active captures provided samples with higher evenness, including some species that were not sampled through baited traps. This analysis allows concluding that baited traps and active capture are complementary sampling methods for the collection of Calyptratae, and specifically for sarcosaprophagous species. From the point of view of biodiversity, the combination of both methodologies allows for a more complete inventory of sarcosaprophagous flies in the temperate forests of austral South America. Indeed, baited traps with putrescine were a very efficient method to sample sarcosaprophagous Calyptratae, due to its high specificity, diversity, and capture rate recorded. Thus, it can be concluded that this method is adequate when the aim is to analyze sarcosaprophagous species with potential medical impact. However, when the aim is to perform biodiversity inventories, a combination of the three techniques is more recommendable, especially in the case of biologically complex families (e.g., Anthomyiidae, Muscidae and Sarcophagidae), and especially when the inventory is framed with a taxonomic perspective.

Acknowledgments

We gratefully acknowledge the financial support from CONICET and ANPCyT [PIP 11220090100548 "Biodiversidad, Sistemática y Conservación de Sarcophagidae y Muscidae (Diptera: Calyptrata) en la Patagonia Septentrional" and PICT 2012-0231 "Análisis de la biodiversidad de las Calyptratae (Diptera), un grupo de insectos hiperdiverso en remanentes de pastizal pampeano"]. We are grateful to the personnel of "Administración de Parques Nacionales" for their valuable logistical support during the field work.

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