ECOLOGY, BEHAVIOR AND BIONOMICS

Evaluation of Three Methods for Sampling Ground-Dwelling Ants in the Brazilian Cerrado

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Neotropical Entomology 37(4):399-405 (2008)

Avaliação de Três Métodos para Amostragem de Formigas do Solo no Cerrado Brasileiro

RESUMO - Poucos estudos avaliaram a eficiência de métodos para a coleta de formigas, especialmente em regiões com fisionomias vegetais bastante variáveis como a do Cerrado. Neste trabalho, foram comparados três métodos para a coleta de formigas do solo: armadilhas de solo, iscas de sardinha e o extrator de serapilheira de Winkler, com o objetivo de determinar o mais apropriado para caracterizar as assembléias de formigas associadas a diferentes tipos de vegetação. Mais espécies foram coletadas com armadilhas de solo e com o extrator de Winkler do que com iscas. As armadilhas de solo coletaram mais espécies nas fisionomias de cerrado (savânicas), particularmente naquelas com pobre cobertura de serapilheira, enquanto o extrator de Winkler foi mais eficiente nas fisionomias florestais, com exceção daquela sujeita a inundações periódicas. Houve baixa similaridade na composição de espécies entre as fisionomias de cerrado e florestais, e esse padrão foi observado com qualquer dos três métodos de coleta. Portanto, mesmo o uso de um único método pode ser suficiente em estudos que comparam condições ou hábitats bastante distintos. Entretanto, se o propósito da amostragem for produzir um inventário mais completo, sugerimos o uso de uma combinação de métodos, em particular as armadilhas de solo e o extrator de Winkler. Desse modo, o Protocolo para Amostragem de Formigas da Serapilheira (*ALL Protocol*) parece ser adequado para a amostragem de formigas na ameaçada região do Bioma Cerrado.

PALAVRAS-CHAVE: Inventário de formigas, isca, Formicidae, armadilha de solo, extrator de Winkler

ABSTRACT - Few studies have evaluated the efficiency of methods for sampling ants, especially in regions with highly variable vegetation physiognomies such as the Cerrado region of central Brazil. Here we compared three methods to collect ground-dwelling ants: pitfall traps, sardine baits, and the Winkler litter extractor. Our aim was to determine which method would be most appropriate to characterize the ant assemblages inhabiting different vegetation types. More species were collected with pitfall traps and with the Winkler extractor than with sardine baits. Pitfall traps collected more species in the cerrado (savanna) physiognomies, particularly in those with a poor litter cover, whereas the Winlker extractor was more efficient in the forest physiognomies, except the one subject to periodic inundations. There was a low similarity in species composition between forest and cerrado physiognomies, and this pattern was detected regardless of the method used to sampling ants. Therefore, even the use of a single, relatively selective method of collection can be enough for studies comparing highly distinct habitats and/or conditions. However, if the purpose of the sampling is to produce a more thoroughly inventory of the ant fauna, we suggest the use of a combination of methods, particularly pitfall traps and the Winkler extractor. Therefore, the Ants of the Leaf-Litter (ALL) Sampling Protocol appear to be an adequate protocol for sampling ants in the highly-threatened Brazilian cerrado biome.

KEY WORDS: Ant inventory, bait, Formicidae, pitfall trap, Winkler extractor

Ants are an abundant, diverse, and ecologically important group of insects in tropical and sub-tropical ecosystems throughout the world (Hölldobler & Wilson 1990). In addition, because ants are responsive to changing environmental conditions and relatively easy to sample and identify, they have been frequently used for conservation assessment purposes, to monitor environmental impact, ecosystem management, and the recovery of ecosystems (Majer 1983, Folgarait 1998, Andersen & Majer 2004). In these studies, as well as in many other ant ecological studies, different treatments, sites, or habitats are compared using replicated and random sampling of the ant community.

Many methods have been developed to sample ants and each one has its own advantages and limitations (Bestelmeyer *et al*. 2000). The choice of a method or methods usually depends on the nature of the study (Romero & Jaffe 1989, Agosti & Alonso 2000). Recently, however, the use of standardized methods by different researches have been advocated in order to allow more strict comparison of the individual studies that could therefore be placed into a larger context (Agosti & Alonso 2000). A protocol for the collection of ground-dwelling ants, the Ants of the Leaf-Litter (ALL) Protocol, was proposed (Agosti & Alonso 2000) and successfully applied in some studies (Fisher *et al*. 2000). However, all these studies were conducted in tropical forests, and therefore it is not clear if the ALL protocol is adequate for ant sampling in non-forest habitats, where other protocols might be more appropriate (Romero & Jaffe 1989, Parr & Chown 2001).

The region of the Cerrado in central Brazil is covered by a mosaic of vegetation types, which include mostly savannas of very variable structure (collectively know as cerrado *sensu lato*) on the well-drained interfluves, and forests along the water courses or on areas of richer soils (Oliveira-Filho & Ratter 2002). Studies of ground-dwelling ant communities in the cerrado are relatively scarce, and the existing studies have relied exclusively on baits to sample ants (Silvestre & Brandão 2000, Silva *et al*. 2004, Marques & Del-Claro 2006), so there is no comparative information on how efficient other sampling methods are. In addition, the studies conducted so far have focused only on the cerrado (savanna) physiognomies, and therefore it is not clear if a method adopt to sample ants in the cerrado is also appropriate for comparative studies with the nearby forest physiognomies.

Here we evaluated the efficiency of three methods to sample ants in different types of vegetation in the Brazilian Cerrado. The methods evaluated included the Winkler litter extractor, pitfall trapping, and sardine baiting. The first two methods are those adopted in the ALL protocol (Agosti & Alonso 2000), while sardine baiting has been extensively used in the Brazilian savannas (Silvestre & Brandão 2000, Silva *et al*. 2004, Vasconcelos & Vilhena 2006).

Material and Methods

Study site. The study was conducted at the Reserva Ecológica do Panga, with 404 ha, located 30 km south of Uberlândia, in the state of Minas Gerais, Brazil (19º10'S, 48º23'W). The region is characterized by a subtropical climate with two well-defined seasons: a dry winter (May to September) and a rainy summer (October to April). The mean annual temperature and precipitation are 22ºC and 1650 mm, respectively. Soils at the site are primarily red latosols. The reserve contains most of the plant physiognomies typical of the Cerrado region. Most of the reserve is covered by cerrado physiognomies - from which cerrado *sensu stricto* is the dominant one - , but forest physiognomies are also found, especially along the stream valleys. For a more detailed description of the vegetation of the study area see Schiavini & Araújo (1989).

Ant sampling. Ants were collected in six transects scattered over the cerrado and forest physiognomies. In the former, three transects were established, one in the campo cerrado, one in the cerrado *sensu stricto* and one in the cerrado denso. These three physiognomies represent a gradient of increasing tree and leaf-litter cover and, consequently, a decrease in grass cover (Oliveira-Filho & Ratter 2002). In the forest physiognomies, we established one transect in the semi-deciduous forest and two transects in the gallery forest. One of the transects in the gallery forest was in a steep slope, whereas the other was in a relatively flat area. As a consequence, the first area remains unaffected by the occasional floods of the adjacent river (Ribeirão do Panga), while the second is invaded by the river water, and when this happens part of the leaf-litter cover is removed by the water flow, leaving many parts of the transect on bare ground. Therefore, hereafter, the second transect is referred to as "gallery forest subject to flooding".

Each transect was 400 m long, and within each transect 20 sampling stations were established as recommend in the ALL protocol (although here we used a spacing of 20 m between stations instead of the suggested 10 m spacing). We used three methods to sample ants in these stations. Ants were sampled using sardine baits, pitfall traps and the Winkler extractor (Bestelmeyer *et al*. 2000). To facilitate location of the ants, we placed each sardine bait on a small piece of white paper and collected any ant found on or under the paper, 30 and 60 min after setting the bait. Collections were performed during the mornings, between 7:30 a.m. and 11:30 a.m, in warm days. The pitfall traps (6.5 cm diameter, 300 ml vol.) were filled to two-thirds of its capacity with soapy water. The pitfall traps operated for a period of 48h, and after this period the ant specimens presented in the traps were sorted and fixed in alcohol. For collections using the Winkler extractor all litter found in 1 m^2 was removed from the ground and sieved through a 0.8 cm mesh. Collections using Winkler were performed at the same time as those using baits. The sifted litter was then transferred to a Winkler extractor (Bestelmeyer *et al*. 2000) which remained in operation for 48h. In each transect, we placed a total of 20 sardine baits, 20 pitfall traps, and collected 20 litter samples, with the exception of the transect in the campo cerrado and the transect in the semi-deciduous forest in which only 14 litter samples were taken. Collections with pitfall traps were performed in late October and early November 2002, those with the Winkler extractor in November 2002, and those with sardine baits in early December 2002.

Ants were sorted to genus and then to morphospecies, or species whenever possible. Voucher specimens are deposited at the Zoological Collection of the Universidade Federal de Uberlândia (UFU), in Uberlândia, MG, Brazil, and at the Myrmecological Collection of the Centro de Pesquisas do Cacau (CEPLAC) in Itabuna, BA, Brazil.

Data analysis. We evaluated the extensiveness of each sampling method by constructing sample-based accumulation curves. The observed number of species was then compared with the number of species estimated to be found, as calculated by three commonly-used species richness estimators: the Jacknife1, the Chao2, and the incidence-based coverage estimator (ICE) (Colwell 2000). For each transect we determined the observed

number of species, the number estimated to be found (through calculation of the species richness estimators) and the rarefied number of species for n = 54. The latter was calculated because the number of samples was not the same in all transects; two transects had less samples (n = 54) than the remaining four transects (n = 60 samples). Sample accumulation curves, rarefaction curves, and estimates of species richness were computed using EstimateS (Colwell 2000).

The similarity in ant species composition between transects was calculated using the Jaccard index of similarity. The resulting values were used to construct similarity dendrograms for comparing the different plant physiognomies as based on ant collections using a single sampling method or a combination of methods. For these analyses we used Systat 10.2 (SPSS 2000).

Results and Discussion

We collected 136 species from 43 genera (a complete list of the species is available from the authors upon request). More ant species were collected in the three cerrado physiognomies combined than in the forest physiognomies combined (Table 1). Similarly, all species richness estimators indicated that the number of species expected to be found in the cerrado physiognomies is greater than the one expected in the forest physiognomies (Table 2). Nevertheless, the number of species observed (as well the number expected to be found) per physiognomy was very similar between forest and cerrado.

From 59 to 72 ant species were collected in each forest physiognomy, whereas in the cerrado this number ranged

Table 1. Number of species within different ant genera collected with three sampling methods in two types of vegetation.

Genus	Sampling method				Vegetation	
	Pitfall	Winkler	Baits	All methods	Forest physiognomies	Cerrado physiognomies
Acanthognathus	0	1	0	1	1	0
Anochetus	1	1	0	1	1	1
Apterostigma	0	1	0	1	1	0
Atta	2	1	1	2	1	2
Brachymyrmex	2	2	2	2	1	2
Camponotus	17	6	13	18	12	16
Cephalotes	2	2	2	3	2	2
Cerapachys	0	1	0	1	1	1
Crematogaster	3	2	4	6	4	2
Cyphomyrmex	2	2	0	2	2	2
Dorymyrmex	1	1	1	1	1	1
Eciton	0	1	0	1	1	0
Ectatomma	6	2	6	6	5	6
Forelius	2	1	0	2	0	2
Gnamptogenys	1	1	0	1	1	1
Hylomyrma	1	2	0	2	1	2
Hypoponera	4	6	0	7	7	2
Labidus	1	0	0	1	0	1
Leptothorax	0	2	0	2	1	2
Linepithema	1	2	0	2	1	2
Megalomyrmex	0	2	0	2	2	0
Mycetagroicus	1	0	0	1	0	1
Mycetarotes	0	1	0	1	0	1
Mycocepurus	2	2	0	2	0	2
Myrmelachista	1	0	1	1	1	1
Myrmicocrypta	1	2	0	2	2	1
Octostruma	1	1	0	1	1	0
Odontomachus	3	2	0	3	2	2

Continue

Table 1. Continuation.

Genus	Sampling method				Vegetation	
	Pitfall	Winkler	Baits	All methods	Forest physiognomies	Cerrado physiognomies
Oligomyrmex	0	1	0	1	1	1
Oxyepoecus	1	0	0	1	0	1
Pachycondyla	4	2	1	4	3	3
Paratrechina	2	2	0	2	2	2
Pheidole	17	21	9	24	17	19
Pogonomyrmex	1	1	1	1	0	1
Prionopelta	0	1	0	1	1	1
Pseudomyrmex	3	1	1	3	3	3
Pyramica	0	1	0	1	1	0
Rogeria	0	1	0	1	1	1
Sericomyrmex	1	1	0	1	1	1
Solenopsis	5	6	3	8	6	6
Strumigenys	2	4	0	4	4	2
Trachymyrmex	7	5	1	7	2	7
Wasmannia	2	2	2	2	2	2
Total	100	96	48	136	96	105

from 61 to 69 (Table 2). Therefore, the higher number of species found in all three cerrado physiognomies combined is probably explained by a higher species turnover among the cerrado than among the forest physiognomies. Two factors may have contributed to this. One of these relates to the distance between sampling sites; the three forest transects were in relative close proximity (range: 30 to 200 m), whereas the cerrado transects were more far apart (800 to 1200 m). The other factor, which we believe is the most important, relates to the greater structural difference among the cerrado physiognomies than among the forest ones. While all forest physiognomies were structurally very similar to each other, the cerrado ones ranged from a very open cerrado to a cerrado with a dense tree cover. Such variation in vegetation structure is not so pronounced in the savannas near Alter do Chão, in the Brazilian Amazon (H. L . Vasconcelos, pers. obs.), and this may help to explain why these savannas support less ant species than the adjacent forests (Vasconcelos & Vilhena 2006), while here the opposite trend was found. The savannas of the Beni Biosphere Reserve in Bolivia were also found to support less ant species than the adjacent forested areas, but this is perhaps because the savannas of the Beni Reserve are periodically inundated whereas the forests are not (Verhaagh & Rosciszewski 1994).

Within the cerrado physiognomies, the number of species tended to increase as tree density and leaf-litter increased, with more species being found in the cerrado denso than in the cerrado *sensu stricto* or the campo cerrado (Table 2). Among

Table 2. Ant species richness in the forest and cerrado physiognomies. Observed number of species, number of species after rarefaction for n = 54, and estimated number of species for the Jacknife1, Chao2, and incidence-based coverage (ICE) estimators. Standard deviations are presented within brackets.

Vegetation physiognomy	Number of ant species				
	Observed	Rarefied	Jacknife1	Chao2	ICE
Campo cerrado	66	66	85.6 (5.4)	74.1 (4.9)	85.9 (2.3)
Cerrado *sensu stricto*	61	58.6 (1.5)	82.6 (5.1)	76.1 (7.9)	83.5 (2.2)
Cerrado denso	69	67.2 (1.6)	96.5. (5.3)	136.4 (12.9)	100.3 (2.6)
Semi-deciduous forest	65	64	86.6 (5.7)	88.8 (11.8)	89.1 (1.9)
Gallery forest	72	69.8 (1.4)	91.7 (4.6)	82.4 (5.8)	87.8. (1.2)
Gallery forest (periodically flooded)	59	56.8 (1.4)	78.7 (5.9)	70.0 (6.1)	80.4 (1.4)
All cerrado physiognomies	105	69.9 (3.9)	135.8 (6.1)	132.2 (11.3)	135.0 (1.6)
All forest physiognomies	96	69.2 (3.5)	114.9 (5.3)	110.7 (8.3)	109.5 (1.4)

the forest physiognomies studied, we found less species in the gallery forest subject to flooding than in the other forest physiognomies (Table 2). This is because flooding probably not only had a negative direct effect on ground-dwelling ants, but also an indirect one since it removed most of the existing litter cover (C.T. Lopes, pers. obs.).

We collected 100 species with the pitfall traps, 96 with the Winkler extractor and 48 with sardine baits (Table 1). In all the six sampling sites, we collected more species using pitfall traps or the Winkler extractor, than using sardine baits (Fig. 1). Pitfall traps collected more species than the Winkler extractor in the campo cerrado, in the cerrado *sensu stricto* and in the gallery forest subject to flooding. On the other hand, more species were collected with the Winkler extractor in the semi-deciduous forest and the gallery forest not subject to flooding (Fig. 1). In the cerrado denso, both the pitfall and the Winkler produced the same number of species. These results support the view that the efficiency of the Winkler litter extractor is dependent on the amount of leaf-litter cover (Parr & Chown 2001).

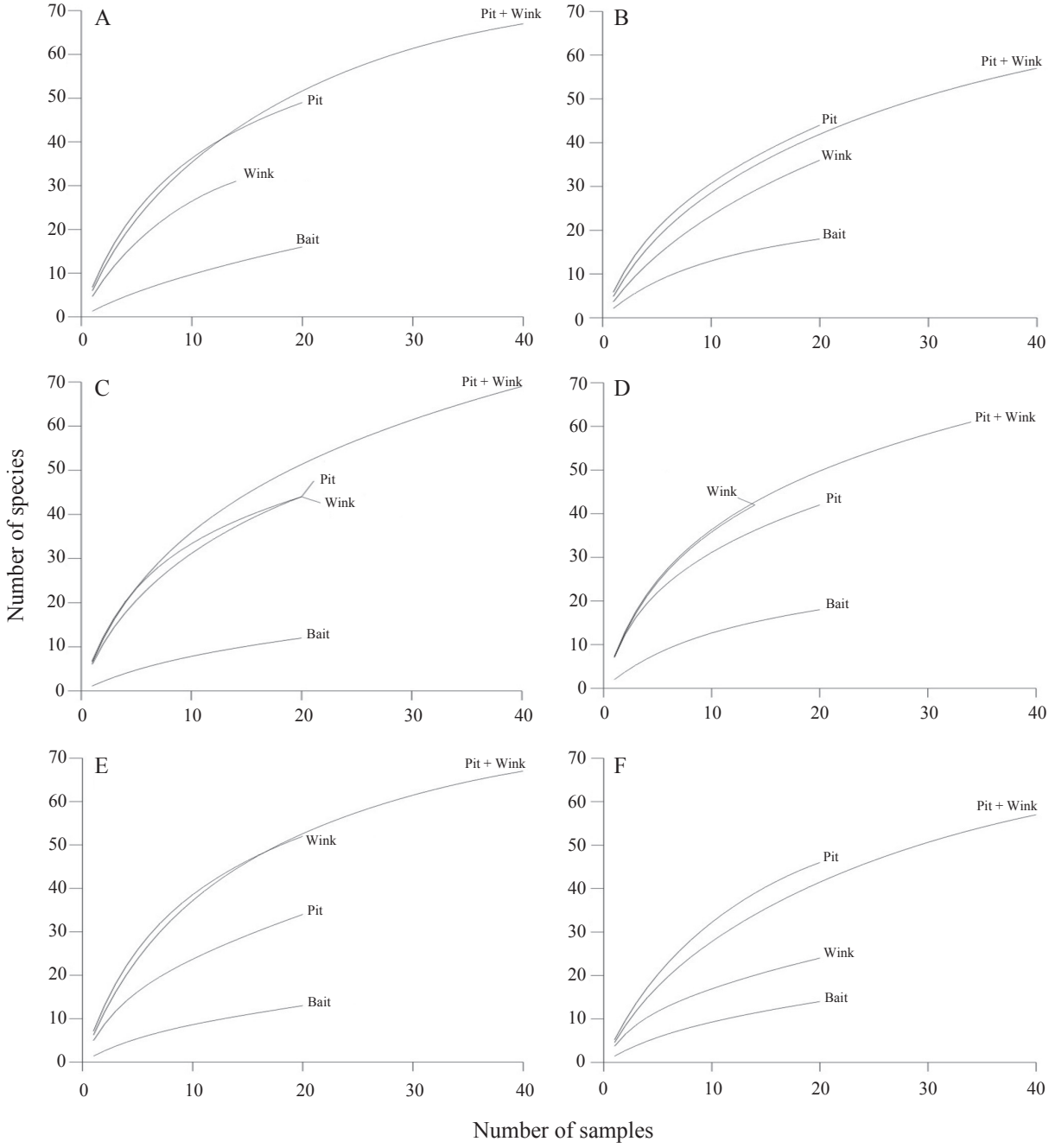

Fig. 1. Species accumulation curves of the number ant species collected, in different plant physiognomies, using baits (bait), pitfall traps (pit), the Winkler extractor (Wink), or with the two last methods combined (Pit + Wink). (A) campo cerrado, (B) cerrado *sensu stricto*, (C) cerrado denso, (D) semi-deciduous forest, (E) gallery forest, (F) gallery forest subject to flooding.

The Winkler extractor was more efficient than the pitfall traps where leaf litter was abundant, whereas in the more open cerrado habitats the reverse was true. However, in contrast to what was found in a South African savanna (Parr & Chown 2001), in the cerrado vegetation there was not a large redundancy between the two ant sampling methods. Even in the more open physiognomy (the campo cerrado), many of the ant species collected with Winkler were different from those collected with pitfalls (Table 1 and Fig. 1). Therefore, the use of the two methods in combination, as proposed in the ALL protocol (Agosti & Alonso 2000), seems appropriate. In fact, rarefaction curves indicate that the number of species collected using pitfall traps and the Winkler extractor in combination was greater or equivalent to the number of species collected using only one of the methods in four of the six habitats sampled here (Fig. 1).

Ant species composition differed greatly between the cerrado and forest physiognomies, and this difference was detected whatever method we used to sampling ants (Fig. 2). Therefore, even the use of a single, relatively selective method of collection such as the sardine baits can be enough for studies comparing highly distinct habitats or

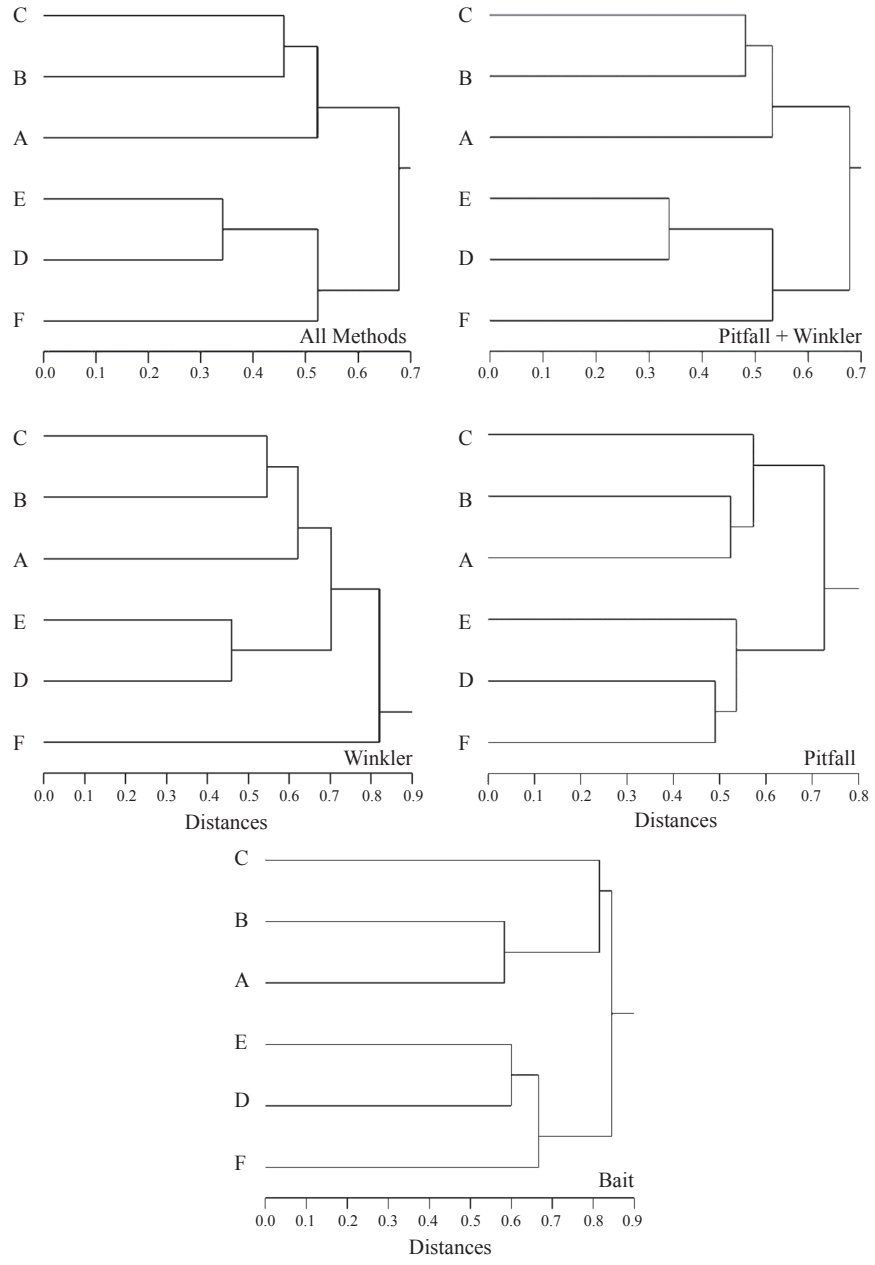

Fig. 2. Dendrograms comparing different plant physiognomies according to the ant species collected using only one or a combination of methods to sample ants. (A) campo cerrado, (B) cerrado *sensu stricto*, (C) cerrado denso, (D) semi-deciduous forest, (E) gallery forest, (F) gallery forest subject to flooding. Distances = 1 – Jaccard index of similarity.

conditions (e.g. Silva *et al*. 2004, Vasconcelos & Vilhena 2006, Vasconcelos *et al*. 2008). However, when differences are more subtle, such as those detected among the forest physiognomies or among the cerrado physiognomies, the use of a combination of methods seems to be preferable. This is because results produced by a single method were not equivalent to those produced by a combination of methods, particularly pitfall traps and the Winkler extractor (Fig. 2) as suggested by the ALL protocol. This protocol, originally developed for study of ants in tropical forests, was recently recommended as a suitable protocol for a through inventory of ants in the temperate and sub-tropical regions of North America (King & Porter 2005). Our study strongly suggests that the ALL protocol is also a good protocol for sampling ground-dwelling ants in the highly-threatened Brazilian Cerrado biome, especially for studies encompassing a variety of habitat types within which the efficiency of pitfall traps and the Winkler extractor has been found to differ greatly.

Acknowledgments

We thank Luciano G. Leone and Alan N. Costa for assistance during field work, Renata Pacheco and Ricardo I. Campos for assistance in sorting the specimens and Jacques Delabie for identifying the ants. Financial support was given by the Universidade Federal de Uberlândia (UFU), the Fundação de Amparo a Pesquisas de Minas Gerais (FAPEMIG CRA-1419/05), and by the Conselho Nacional de Pesquisas e Desenvolvimento Científico e Tecnológico (CNPq research fellowship to HLV).

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Received 14/VI/07. Accepted 29/IV/08.