Richness of Termites and Ants in the State of Rio Grande do Sul, Southern Brazil

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Introduction

Studies on biodiversity are urging in face of diversity loss (Wilson, 1997; McGeoch & Chown, 1998). Termites (Mill, 1982; Constantino, 1992; Eggleton et al., 1995; Black & Okwakol, 1997; Lavelle et al., 1997; Jones, 2000; Gathorne-Hardy et al., 2001; Jones et al., 2003) and ants (Majer, 1983; Hülldobler & Wilson, 1990; Andersen, 1997; Silva & Brandão, 1999; Ward, 2000; Ribas et al., 2012) have been considered biodiversity indicators due to their biological and ecological characteristics. These groups have also been considered as surrogate groups in evaluations of conservation status, degradation, or recovery of terrestrial ecosystems (New, 1996; Majer, 1996; Andersen, 1997; Majer & Nichols, 1998; Lobry de Bruyn, 1999; Jones & Eggleton, 2000; Bandeira & Vasconcello, 2002; Diehl et al., 2004).

Like ants, termites are entirely eusocial and have profound ecological significance in the tropics (Engel et al., 2009). Studies have shown that ants and termites play a crucial role to create soil structure, influence aeration, water infiltration and nutrient cycling acting as ecosystem engineers (Lobry de Bruyn & Conacher, 1990; Lavelle et al., 1997; Mora et al., 2005; Jouquet et al., 2011; Del Toro et al., 2012). In some cases, termites and ants are also responsible for increased crop yield under dry conditions through soil water infiltration due to their tunnels and improved soil nitrogen (Evans et al., 2011).

Environmental disturbances, such as deforestation, forest fragmentation, fire, and floods may affect the community structure of termites (De Souza & Brown, 1994; Eggleton et
al., 1994, 1995; Davies, 2002; Bandeira et al., 2003; Inoue et al., 2006) and ants (Hölldobler & Wilson, 1990; Majer & Nichols, 1998; Diehl et al., 2004; Schmidt & Diehl, 2008). Previous studies have shown that local species richness of ants and termites is influenced by environmental factors, such as temperature, rainfall, and vegetation (Benson & Harada, 1988; Silva & Brandão, 1999; Gathorne-Hardy et al., 2001; Oliveira & Del-Claro, 2005; Del-Claro, 2008; Jones & Eggleton, 2011). There has also been some evidence that increasing altitude and decrease of temperature decrease species richness (Kusnezov, 1957; Collins, 1980; Ward, 2000; Gathorne-Hardy et al., 2001) and that the phylogenetic structure might be involved in this process, specially in the temperate forests (Machac et al., 2011).

The Neotropics rank in the third position in termite richness among the biogeographic regions, with the Oriental and Ethiopian ones presenting the highest number of species recorded so far (Krishna et al., 2013). About 72% of the neotropical species occur in South America (Constantino, 2014) and the sites with good termite surveys are between the parallels 0° S and 20° S, which include the biomes Amazon and Atlantic Forests, Cerrado and Caatinga (Constantino & Acioli, 2006). A review of the termite fauna in across Brazil can be found in Constantino & Acioli (2006). Recent studies recorded 33 species for five fragments of semideciduous Atlantic Forest in northern Brazil, varying locally from 11 to 27 species (Souza et al., 2012). With different levels of perturbation, 26 species were recorded in a region of semi-arid Caatinga (Vasconcellos et al., 2010). In southern Brazil, termite fauna is poorly known. Studies mention only 16 termite species to Rio Grande do Sul (Araújo, 1977; Constantino, 1998; Fontes, 1998; Castro & Diehl, 2003; Diehl et al., 2005b; Florencio & Diehl, 2006). This low number can be due to the climate (Eggleton et al., 1994; Constantino & Acioli, 2006), or lack of taxonomists and local studies (Diehl-Fleig et al., 1995; Constantino & Acioli, 2006).

Despite its importance, little is known about the edaphic insects, specially ants and termites, in southern Brazil. Thus, here we present the richness and a checklist of termites and ants in the four geomorphic units of Rio Grande do Sul, reporting new records for state.

The checklist also includes species from other inventories conducted in the state of Rio Grande do Sul by researchers apart from our group leaded by E. Diehl. Most aimed at collecting species of *Acromyrmex* and *Atta* or urban ant fauna and few have focused on ant species inventories in forests and other relevant ecosystems (please see supplementary material for further details at):

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### Material and Methods

#### Study areas

**Geomorphological units of Rio Grande do Sul**

The southernmost Brazilian state of Rio Grande do Sul has four geomorphologic units: Southern Plateau, Central Depression, Sul-Riograndense Shield, and Coastal Plain (Fig 1). Two main vegetation types originally occurred in the state: forests and grasslands occupying 34% and 46% of the state area, respectively. The remaining was occupied by coastal vegetation, wetlands, and other vegetation types. The vegetation reflects soil type and origin and forests usually occur where the soil is deep and fertile. Grasslands develop where the soil is siliceous or shallow. While the southern region of the state is dominated by grasslands, the northern grasslands are interspersed with Araucaria forest (Rambo, 1994).

The weather in southern Brazil is subtropical humid, according to the Köppen classification. While the average annual temperature in the Coastal Plain is around 18°C, temperature approaches 20°C in the Central Depression, which also has mild winters. Conversely, the annual average temperature in the Southern Plateau does not exceed 15°C, due to its high altitude, winters are harsh, with often negative temperatures, severe frosts, and even deep snow. The Campanha, in the Sul-Riograndense Shield, is an open landscape and receives all continental winds in the winter, but due to their large grassland areas it receives much sunlight resulting in summers with high temperatures (Rambo, 1994).

Sampling sites (Fig 1) are described below and are mostly under different levels of disturbance:

1. remnants of seasonal semideciduous forest and suburban areas around São Leopoldo (29° 45' - 29° 47' S, 51° 05' - 51° 10' W; 15 m a.s.l.) in the Central Depression (Mayhé-Nunes & Diehl-Fleig, 1994; Diehl-Fleig & Fleig, 1997; Haubert et al., 1998; Flores et al., 2002; Diehl et al. 2006; Florencio & Diehl, 2006; Marchioretto & Diehl 2006);
2. periurban and urban areas in Canela (29° 21' S, 50° 49' W; 819 m a.s.l.) and Gramado (29° 22' S, 50° 52' W; 825 m a.s.l.) in the Southern Plateau (Diehl-Fleig, 1997);
3. dunes and restingas in Torres (29° 20' - 29° 23' S, 49° 43' - 49° 46' W; 0 - 16 m a.s.l.) in the northern Coastal Plain (Diehl-Fleig et al., 2000; Hameister et al., 2003; Albuquerque et al. 2005; Diehl, unpub. ms.);
4. grasslands in the Caçapava do Sul (30° 47' S, 52° 24' W; 220 m a.s.l.) in soils with different copper levels, including a copper mine and a native savanna area in the Camaquã River Basin in the Sul-Riograndense Shield (Diehl et al., 2004);
5. restingas in Morro da Grota (30° 21’ S, 50° 01’ W; 263 m a.s.l.) near Lagoa dos Patos and a typical forest of granite...
soil, sandy and rocky areas in Pedreira beach (30° 21’ S, 50° 02’ W; at the sea level), on the banks of the Guaiaba Lake, both at the Itapuã State Park in Viamão, which is within both Sul-Riograndense Shield and Coastal Plain (Sacchett & Diehl, 2004; Diehl et al., 2005a; Diehl, unpub. ms.);

6) Eucalyptus plantation in restingas in Capivari do Sul (30° 11’ S, 50° 21’ W; 12 m a.s.l.) and Tramandai (29° 59’ S, 50° 13’ W; 8 m a.s.l.) in the northern Coastal Plain (Fonseca & Diehl, 2004);

7) wetland used as pastures for cattle or irrigated rice in Santo Antônio da Patrulha (29° 54’ S, 50° 33’ W; 25 m a.s.l.) in the Coastal Plain (Diehl et al., 2005b; Moraes & Diehl, 2009);

8) an area of mixed ombrophilous forest in the São Francisco de Paula National Forest (29° 23’ - 29° 27’ S, 50° 23’ - 50° 25’ W; 930 m a.s.l.) and in the Pró-Mata Research and Nature Conservation Center (29° 28’ S, 50° 13’ W; 900 a.s.l.) in the Southern Plateau (Diehl et al., 2005c; Pinheiro et al., 2010);

9) vineyards (29° 56’ S, 51° 33’ W; 645 m a.s.l.) in Bento Gonçalves, within the northeastern Central Depression (Sacchett et al., 2009);

10) yerba mate plantations in Mato Leitão (29° 31’ S, 52° 07’ W; 81 m a.s.l.), Ilópolis (28° 55’ S, 52° 07’ W; 683 m a.s.l.), and Putinga (29° 00’ S, 52° 09’ W; 435 m a.s.l.) in the northeastern Central Depression (Junqueira et al., 2001; Steffens, 2006);

11) primary forest and reforestation area in Alto Ferrabraz, Sapiranga (29° 34’ S, 50° 56’ W; 500 m a.s.l.). This is a transition zone between the Southern Plateau and the northeastern Central Depression (Haubert, 2006);

12) secondary forest, with Acacia plantation and an area with reforestation in Rolante (29° 36’ 32.2” S, 50° 31’ 39.1” W; 370 m a.s.l.) in the Central Depression (Schmidt & Diehl, 2008);


At each termite sampling site, we established a transect (100 m x 3 m), further divided into 20 sections of 15 m². We then sampled ten non-contiguous sections on the right and left sides of the transect alternately, with a sampling effort of 60 minutes per section. In each section, we search for termites in leaf litter and humus at the base of trees and roots, under both rocks and fallen trees, inside logs, hollow twigs, decomposing fallen branches, epigean and arboreal nests. We also set up one TermiTrap® bait per section (Almeida & Alves, 1995) at 15 cm deep, 60 days before samplings. Furthermore, we also searched for termites in ten blocks (340 cm³) of soil horizon A per section.

Termites were stored in individual amber glass with 80% ethanol. Termites were identified to the genus level by Fontes, 1995; Constantino, 1999; 2002) and eventually to the species level or morphospecies. We also consulted specialist taxonomists to confirm the identification of termites.

We used five techniques to sample ants: 1) manual capture of all ants on the ground, under stones, in hollow trees, and branches; 2) baits with sardines in vegetable oil on a piece of filter paper (6 cm²); 3) leaf litter samples, firstly sieved in the field and further using Winkler extractors, in which they remained for 72 hours; 4) pitfall traps with and without attractive, which consisted of 200-300 mL plastic cups buried up to the top containing 70% ethanol, remaining on the ground for 24 to 48 hours. Attractive traps were composed by sardines in vegetable oil pierced in a galvanized wire rod; 5) underground trap with attractive, which was a small plastic pot (3.3 cm wide by 5 cm high) with small holes on the sides. Within each trap a piece of 1 cm³ sardines in vegetable oil was added. Traps were buried 20 cm deep by 48 hours. In grasslands we used direct sampling, sardine baits and pitfall traps. In dunes, we only used direct sampling and sardine baits. At each site, we established two to three 100 m transects, along which we sampled every 10 m. Sampling effort was at least 2 hours in each site.

We classified ants into subfamilies following Bolton (2014) and by genus following Bolton (1994) and Fernández (2003). The subfamilies Ponerinae and Ecitoninae were treated according to the most recent reviews - see supplementary material for details. We used several identification keys and also consulted specialists to identify species. We assigned specimens to morphospecies when specific identification...
was not possible. At least three specimens of each species/morphospecies were mounted with entomological pins and the remaining preserved in 70% ethanol.

Specimens of termites and ants are housed at the Collection of Social Insects (Isoptera and Formicidae) of the Universidade do Vale do Rio dos Sinos (UNISINOS), São Leopoldo, Rio Grande do Sul, Brazil.

The checklist presented results of ca. 30 years of collections leaded by E. Diehl added to most ant inventories conducted by few other researchers in the state of Rio Grande do Sul. We reviewed papers published from 1972 to 2014 included in the databases ISI Web of Science, Scielo and Google Scholar that contained the keyword combination: ant OR Formicidae AND Rio Grande do Sul. Only papers referring nominal species and site location (municipality or more specific location) were considered, except those with doubtful identification (approximate identification, to be confirmed, compared to, of group, subgenus or species complex). Three studies on Atta and Acromyrmex distribution were excluded because site location was not provided. Species previously registered to the state was provided by the catalog of Kempf (1972) with additions of Brandão (1991).

Results

We recorded 16 isopterans from two families (Table 1). We only found one species of the Kalotermitidae genus Rugitermes. We also recorded a new genus of Termitidae, besides seven morphospecies and seven species from four subfamilies (Apicotermitinae, Nasutitermitinae, Syntermitinae, and Termitinae).

Termitidae contained the highest richness. We recorded 10 species in the Southern Plateau (181-1,200 m a.s.l.) and 15 in the Central Depression (20-30 m), whereas in the Sul-Riograndense Shield (23-29 m) and northern Coastal Plain (5-15 m) we found the lowest richness: four and six species, respectively.

We found 26 ant genera from seven subfamilies (Ectatomminae, Dorylinae, Ectatomminae, Formicinae, Myrmicinae, and Ponerinae) that were stored in 70% ethanol. Furthermore, we recorded 51 genera from nine subfamilies (Amblyoponinae, Dolichoderinae, Dorylinae, Ectatomminae, Formicinae, Heteroponerinae, Myrmicinae, Ponerinae, and Pseudomyrmecinae), which were stored in entomological drawers. In total, we recorded 73 species and 115 morphospecies in Rio Grande do Sul. Compiling only nominal species, we recorded 108 species and 105 morphospecies from the catalogs (Kempf, 1972; Brandão, 1991), from our research group and the other groups. This number would likely increase, if we consider the morphospecies not yet identified to the species level and other published studies with nominal species and site location indicated (see study materials available as supplementary material).

Ant richness and composition varied along the geomorphologic units. We found 88 species/morphospecies in the Southern Plateau (900-1,200 m a.s.l.), 40 in the transition between the Southern Plateau and the Central Depression (500 m), 108 in the Central Depression (10-683 m), 51 in the Sul-Riograndense Shield (220 m), 81 in the transition between the Sul-Riograndense Shield and the Coastal Plain (16-263 m), and 97 in the Coastal Plain (0-80 m). Of the 188 species/morphospecies, most (40.43%) were restricted to a single geomorphologic unit, either by 2.5% were common to all units (Pheidole sp.3, Pheidole sp.6, Pheidole sp.15, Wasmannia sp. and Wasmannia sp.1).

Excluding three sites where ant fauna was inventoried exclusively in agricultural or urban areas, there is some indication that species richness and composition could vary in response to an altitudinal gradient. The highest ant richness, 56, 81, and 73 species were found in sites at the sea level, 10 m, and 26 m, respectively. Conversely, we found 51, 35, 39, 55, and 33 species in sites at 220 m, 370 m, 500 m, 930 m, and 1,200 m, respectively. Of the 158 ant species/morphospecies in these eight areas, none were present in all sites, only ca. 3% were common to seven areas. The great majority (35.44%) of ants were restricted to a single site.

| Table 1. Termites recorded in the state of Rio Grande do Sul, Brazil. |
|--------------------------------|------------------|
| **Family/Subfamily** | **Taxon** |
| Kalotermitidae | Rugitermes sp. (1, 3, 4)* |
| Termitidae | Apicotermitinae 1 (1, 2, 3, 4) Apicotermitinae 2 (1, 2, 3, 4) Grigiotermes bauerni (Snyder & Emerson, 1949) (3) Grigiotermes sp. (3) Ruptitermes sp. (3) Tetintermes sp. (3) New Genus 1 (3) |
| Nasutitermitinae | Araujotermes caissara Fontes, 1982 (1) Cortaritermes fulviceps Silvestri, 1901 (1, 2, 3, 4) Nasutitermes aequilinuus Holmgren, 1910 (3, 4) Nasutitermes jaraguensis Holmgren, 1910 (3, 4) |
| Syntermitinae | Cornitermes cumulans Kollar, 1832 (2, 3, 4) |
| Termitinae | Dihoplotermites insitutius Araújo, 1961 (3, 4) Neocapritermes sp. (1, 3, 4) Termes sp. (3, 4) |

*Geomorphologic units: 1, Coastal Plain; 2, Sul-Riograndense Shield; 3, Central Depression; 4, Southern Plateau
Discussion

According to Krishna et al. (2013) there are 2,937 termite species worldwide, of which 569 are found in the Neotropics. The On-line Termite Database maintained by Constantino (2014) refers 2,882 termite species in the world, of which 562 species occur in the Neotropics (database was last updated in September 2012). Estimates of termite richness for Brazil are not yet accurate, ranging from 250 to 364 species (Constantino & Schlemmermeyer, 1999; Constantino, 1999; 2014; Fontes & Araújo, 1999). However, the estimated species richness should increase with termite sampling in other areas of the country. Our results provide records of a new genus likely belonging to Apicotermitinae, another unidentified species of this subfamily (Apicotermitinae 1), and Diholotermes insititatus. Thus, the number of known termite species in Rio Grande do Sul increases to 19.

Drywood termites of the family Kalotermitidae have major economic importance and a wide geographic distribution. These termites are poorly known because they inhabit places difficult to access (Jones & Eggleton, 2011). The largest and most diverse isopeteran family is Termitidae (Eggleton & Tayasu, 2001). We found species from four out of the eight termite subfamilies previously known to occur in Rio Grande do Sul (Apicotermitinae, Nasutitermitinae, Syntermitinae, and Termitinae). A negative correlation between altitude and species richness has been reported for various organisms (Rahbek, 2005; McCain, 2009; McCain & Grytnes, 2010). This correlation seems to be dependent on altitude in tropical and subtropical areas. Altitude is positively correlated with richness in latitudes below 30° and below 500 m, but negative in latitudes above 30° and at altitudes above 500 m (Kusnezov, 1957; Ward, 2000).

We found indication that termite species richness relates positively to altitude. Previous studies (Inoue et al., 2006) found the species richness of Nasutitermitinae increased with altitude, while species richness and abundance of Macrotermiteae decreased with altitude. The other groups were apparently not influenced by altitude. Recently, Palín et al. (2010) evaluated the influence of the variation along an Amazon–Andes altitudinal gradient in Peru on richness, abundance, and diversity of functional groups of termites. They registered 49 species and verified that, in general, the diversity declined with increased elevation. The functional groups responded differently to the upper distribution limit: for the soil-feeding it was between 925 and 1,500 m a.s.l., while the wood-feeding termites was between 1,550 and 1,850 m a.s.l. And this differential response led the authors to suggest that the energy requirements for each group are a key factor in shaping their occurrence associated with the altitude and temperature.

Termite species richness seems to be influenced by local environmental conditions, such as altitude, temperature, rainfall, and vegetation (Collins, 1980; Gathorne-Hardy et al., 2001; Davies, 2002; Inoue et al., 2006; Jones & Eggleton, 2011). The species richness of termites we found is as low as reported in subtropical and temperate areas. For example, previous studies found two to eight morphospecies in the Atlantic Forest of southeastern Brazil, whereas an average of 30 morphospecies were found in tropical forests near Bahia, northeastern Brazil (Constantino et al., 2002).

The far southernmost part of South America has often been considered as a separate biogeographic sub-region. It is depauperate in termite species due to its high latitude (Eggleton, 2000), and countries like Chile and Uruguay have even fewer species registered: six and nine, respectively, while 37 are reported for Paraguay (Constantino, 2014). Argentina, on the other hand, has 57 species reported (Constantino, 2014), even though this number could increase to 95 (Torales et al., 2008). Argentina local termite species varies from 12 in the Chaco province (Godoy et al., 2012) to 26 species in northeast Argentina (Laffont et al., 2004).

We present the first two records of the rare, monotypic genus Diholotermes to Rio Grande do Sul. A colony of D. insititatus was found inhabiting a nest Cornitermes bequaerti in gallery forests in Mato Grosso (Mathews, 1977), whereas other studies (Constantino, 1999) found D. insititatus in the Cerrado and disturbed habitats in the Southeast. We found the first record of D. insititatus within a nest of C. cumulans in grasslands surrounding the São Francisco de Paula National Forest. However, we could not find this species in further surveys. This is a rare species that could have went locally extinct after a Pinus plantation covered the area. The other record of D. insitatius was also in a nest of C. cumulans in the suburb of São Leopoldo, more than 500 km away from the first site. With the increase of urbanization in the region, this rare species is subject to rapid local extinction.

The family Formicidae comprises more than 15,700 species and subspecies (AntWeb, 2013); recent estimates suggest that this number should exceed 23,000 species (AntWeb, 2013). However, previous studies mention 208 (Kempf, 1972) or 224 species Brandão (1991) to Rio Grande do Sul. Since its last catalog update (Brandão, 1991), 23 years ago, our study is the first to present an updated checklist of the ant species in Rio Grande do Sul. If we take into account the determination of species level of all morphospecies in our collection at Unisinos and in other studies, the species list would likely increase. Only few list are available for other states, such as Santa Catarina (Ulysséa et al., 2011), where ant richness (366 species and 17 subspecies) is greater than the 265 species now updated to Rio Grande do Sul. Apart from different collection efforts, historical aspects linked to the myrmecology development are part of this numerical difference reported.

Ant inventories in Rio Grande do Sul until the early 2000s focused primarily in the genera Atta and Acromyrmex (e.g., Diehl-Fleig, 1997). This attines have long been known for their pest status and major impact on agriculture, fairly
common and economically relevant throughout the state. It is clear that ant fauna inventories in RS are still very scarce and the geomorphic units are undersampled, particularly the Pampa biome. This grassland ecosystem is unique in fauna and vegetation among Brazilian biomes and less than 42% remains preserved (Roesch et al., 2009). So far, ant fauna has been surveyed mostly in the Atlantic Forest biome in the state (Diehl et al., 2005c; Albuquerque & Diehl, 2009; Pinheiro et al., 2010) while a single study included the Pampa (Marques & Schoereder, 2014).

The diversity of habitats, vegetation, altitude, latitude, and climate among sampling sites should be the main factors responsible for the differences found in termite and ant species richness along the study areas and also other tropical areas in Brazil (Oliveira & Del-Claro, 2005). Currently, 106 ant genera are known to occur in Brazil (Antwiki, 2013), while the termite diversity is much lower, only 74 genera (Constantino, 2014). As expected, we found a higher richness of ant genera than termite along the studied areas. Species diversity decreases with increasing distance from the equator (Kusnezov, 1957; Eggleton et al., 1994; Ward, 2000). Therefore, the lowest species richness of termites and ants found by us was expected. Additionally, the low richness may be due to the subtropical climate, the low latitude (30° S), and high altitudes (> 500 m), as might be case for our findings that ant richness was lower at higher altitudes. Moreover, sampled sites varied in the degree of habitat complexity, from single crop to native forest, which also play a key role in shaping species richness and composition with different outcomes (e.g., Lassau & Hochuli, 2004; Silva et al., 2007; Pacheco & Vasconcelos, 2012). This study is unique in compiling the richness of both termites and ants so far known in the state of Rio Grande do Sul and we hope it provides a landmark in exposing the necessity for future studies and establishing efforts in unraveling our eusocial insect diversity.

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