

## Land flatworm community structure in a subtropical deciduous forest in Southern Brazil

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**ABSTRACT.** Due to their biological characteristics and habitat requirements, land planarians have been proposed as indicator taxa in biodiversity and conservation studies. Herein, we investigated spatial patterns of land flatworm communities in the three main existent vegetation types of the most significant remnant of the subtropical deciduous forest in south Brazil. The main questions were: (1) How species-rich is the study area? (2) How are community-structure attributes allocated in areas with distinct floristic composition? (3) What are the effects of soil humidity and soil organic matter content on flatworm abundance? (4) Are there seasonal differences regarding species richness? (5) Are the communities in the three types of vegetation distinct? Twenty-two flatworm species, distributed in five genera and two subfamilies, were recorded. Results indicated that: (1) species richness, evenness, abundance, and dominance in the three vegetation types are not significantly different; (2) diversity indices were higher for areas with caducifolious forest than for areas with jaboticaba trees with marginally significant differences; (3) flatworm abundance is negatively related to soil organic matter; (4) there are no significant differences in flatworm species richness throughout the year; and (5) flatworm communities in the three types of vegetation are not distinct. Soil organic matter content was abundant at sites with distinct vegetation types, thereby negatively affecting flatworm abundance. The original vegetation of the study area has been well-preserved, which may be an explanation for the absence of a clear separation of the flatworm communities of the three main types of vegetation in the study area.

**KEY WORDS:** diversity, conservation unit, spatial patterns, soil organic matter contents, triclads

### INTRODUCTION

Due to their biological characteristics and habitat requirements, land planarians have been proposed as indicator taxa in biodiversity and conservation studies (SLUYS, 1998). They are top predators within the soil ecosystem, preying on other invertebrates (DU BOIS-REYMOND MARCUS, 1951; OGREN, 1995; JONES & CUMMING, 1998; SLUYS, 1999; LEAL-ZANCHET & CARBAYO, 2001; CARBAYO & LEAL-ZANCHET, 2003; PRASNISKI & LEAL-ZANCHET, 2009). As land flatworms are not adapted to conserve water (KAWAGUTI, 1932), they are sensitive to the humidity and temperature conditions of their environment (FROEHLICH, 1955; WINSOR et al., 1998; SLUYS, 1998, 1999).

From studies on land flatworm community structure, there are indications that they are sensitive to replacement of the natural habitat by plantations with exotic species. Their diversity is inversely related to the degree of habitat disturbance (CARBAYO et al., 2001, 2002; LEAL-ZANCHET et al., 2006; FONSECA et al., 2009). After having studied land flatworm community structure in two types of ombrophilous forest, FICK et al. (2006) showed that the communities were clearly distinct, and suggested that they are not affected by the edge-effects of their habitats.

Investigations on land planarian diversity in forest ecosystems have been concentrated in areas of ombrophilous forest located in the northeast of southern Brazil (LEAL-

ZANCHET & CARBAYO, 2000, 2001; CARBAYO et al., 2001, 2002; FICK et al. 2003, 2006; PALACIOS et al., 2006; BAPTISTA et al., 2006; LEAL-ZANCHET et al., 2006; LEAL-ZANCHET & BAPTISTA, 2009). These studies have registered high species richness, much of it represented by new taxa (LEAL-ZANCHET & CARBAYO, 2001; CARBAYO & LEAL-ZANCHET, 2001, 2003; FROEHLICH & LEAL-ZANCHET, 2003; BAPTISTA & LEAL-ZANCHET, 2005; LEAL-ZANCHET & FROEHLICH, 2006; LEMOS & LEAL-ZANCHET, 2008). In deciduous and semi-deciduous forests, land flatworm communities have been studied only in small remnants located in the central and northeastern regions of the state of Rio Grande do Sul (Southern Brazil), respectively. In those areas, relatively low species richness has been registered (CASTRO & LEAL-ZANCHET, 2005; ANTUNES et al., 2008).

In the past, the largest forest-type in southern Brazil consisted of deciduous forest. What remains now occupies only 23.8% of the originally occupied area and is mainly located in the northwestern and central regions. According to IRGANG (1980), the northwestern part, corresponding to 56% of the total area, represents the sole significant remnant. It is represented by the Turvo State Park, the largest wholly-protected regional conservation unit, being entirely disconnected from the other forest remnants (IBGE, 1986; SEMA/DEFAP, 2005). This Park is also the main wildlife refuge in the region, sheltering a rich fauna. It includes all the large felids that are characteristic to meridional Brazil

(LEMA, 1980; KASPER et al., 2007). Nevertheless, there is a lack of data on fauna community structure, giving rise to concern as to its conservation (ALBUQUERQUE, 1977, 1985; KASPER et al., 2007).

Tropical and subtropical deciduous forest biomes developed in response not to seasonal temperature variations, but to seasonal rainfall patterns. In moist deciduous forests, overall rainfall is heavy, with a warm and wet summer and cooler temperatures in the winter. Some trees lose their leaves during the cooler winter. Typically, trees do not stand as close together as in tropical rainforests, thus facilitating the penetration of sunlight, especially when deciduous species are without leaves. This penetration of sunlight down to the forest floor gives rise to dense undergrowth.

In the present study, we investigated the land flatworm community in the most significant remnant of deciduous forest in southern Brazil, the Turvo State Park. Our main research questions were: (1) How species-rich is the study area; (2) How are community-structure attributes allocated in areas with distinct floristic composition; (3) Is flatworm abundance affected by soil humidity and soil organic matter content; (4) Are there seasonal differences regarding species richness; (5) Are the communities in the three types of vegetation distinct?

## MATERIALS AND METHODS

### Study area

The Turvo State Park (Fig. 1) is located in the northwest of the state of Rio Grande do Sul, southern Brazil (27°00'–27°20'S; 53°40'–54°10'W), and covers an area of

17,491.40ha. It is bounded in the north by the Uruguay River, in the south by private landholdings as well as the Calixto and Bonifácio Rivers, in the east by the Parizinho River, and in the west by the Turvo River. Coverage consists mainly of deciduous forest (IBGE, 1986), with well-developed areas of primary vegetation made up of more than 700 species belonging to 121 families, predominantly the Bignoniaceae, Asteraceae, Euphorbiaceae, Myrtaceae, Fabaceae, and Rubiaceae. The structure of the south Brazilian deciduous forest is represented mainly by two distinct tree strata. The emergent stratum is formed by deciduous trees with heights ranging from 16 to 30 meters. The second stratum is constituted mainly of evergreen species with heights ranging from 10 to 16 meters. In addition, there is a third stratum of young, low trees (IBGE, 1986; VASCONCELOS et al., 1992; A. Backes, personal communication). The physiognomy of this type of deciduous forest is defined by a canopy dominated by caducifolious species (IBGE, 1986). The Turvo State Park also contains areas of secondary vegetation, which were formerly used for farming, occurring in the northwestern and northeastern parts. These are characterized by thinner and smaller trees and a dense understory. There are also large areas in the Park with jaboticaba trees (*Myrciaria trunciflora* Berg), characterized by an understory with few seedlings. They occur in areas with insufficiently drained, very humid soils (SEMA/DEFAP, 2005).

The climate is warm-temperate (subtropical) and humid, without any marked dry periods (NIMER, 1989). The average annual precipitation is about 1,900mm year<sup>-1</sup>, with the heaviest rains occurring in the summer (SEMA/DEFAP, 2005). Soils are mainly clayey, dark-red in color, shallow and quickly drained.

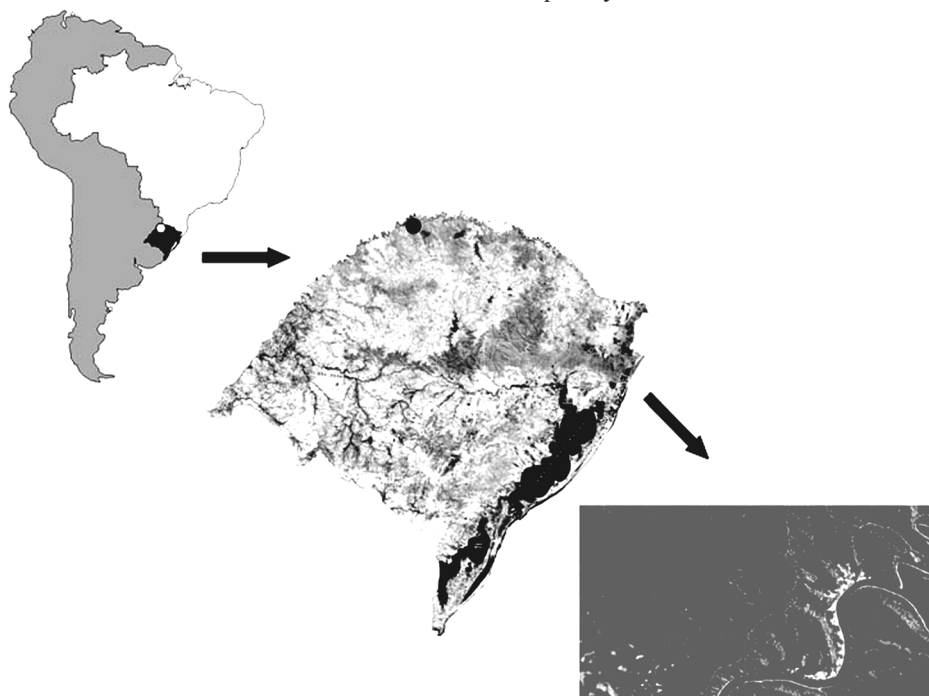


Fig. 1. – Location map of the Turvo State Park in southern Brazil and sampling sites. II-III: areas with jaboticaba trees; PI-PIII: areas with primary vegetation (deciduous forest); SI-SIII: areas with secondary vegetation (deciduous forest).

## Methods

Surveys were carried out in three sites of the following types of vegetation: (1) areas of caducifolious forest with primary vegetation (PI, PII, and PIII) located in the north-east, northwest, and close to the center of the Park, respectively; (2) areas of caducifolious forest with secondary vegetation (SI, SII, and SIII), all located in the southwest; and (3) areas with jaboticaba trees (JI, JII, and JIII), located approximately in the southeast (JI and JII) and central regions (JIII) (Fig. 1). Samples were taken by five experienced collectors, twice per season, from May 2005 through November 2006, amounting to a total of eight samplings. Each sampling lasted three days. In each site ten plots (50m x 2m) were set up. Five of them were randomly selected for each sampling. We searched, at daylight, for flatworms by means of direct sampling in soil litter, under and inside fallen logs and branches, and under rocks. The sampling effort was 1 hour per plot. After inspection, the branches, logs, and rocks were returned to their original positions, in order to avoid alteration of soil microhabitats (BALL & REYNOLDS, 1981; WINSOR, 1997).

We registered the relative humidity of the air at each site with a hygrometer, avoiding rainy days, from October 2005 to November 2006. Soil samples (60g) were collected from

each site by means of a pycnometer in order to analyse humidity and organic matter content. This was done once in March 2006.

For identification we used techniques described by LEAL-ZANCHET & CARBAYO (2001) and SEITENFUS & LEAL-ZANCHET (2004). Adult specimens without all the necessary morphological characteristics required for identification at the genus level were placed in the collective group *Pseudogeoplana* OGREN & KAWAKATSU, 1990. Specimens registered as "unidentified" were immature specimens or animals lost before fixation.

Abundance, species richness, dominance, evenness, and the Shannon diversity index ( $H'$ , see KREBS, 1989) for each area were calculated and the data obtained were log-transformed (log-linear). The ANOVA, followed by Tukey, was applied for comparing habitats. Repeated measures ANOVA was used to verify seasonal distribution of richness. Both forms of variance analysis were calculated using Systat 11.0 (Systat Software Inc., Richmond, OR, USA). Unidentified specimens were considered only for abundance analysis. Collector's curve based on the species accumulated per sampling and the probable number of species (Jackknife Estimators of Species Richness) were calculated using PC-ORD (McCUNE & MEFFORD, 1999).

TABLE 1

Abundance of land flatworms in areas with jaboticaba trees (JI-III), areas with deciduous forest (primary vegetation) (PI-III), and areas with regenerating deciduous forest (secondary vegetation) (SI-III) in the Turvo State Park, southern Brazil.

| Species                       | PI | PII | PIII | SI | SII | SIII | JI | JII | JIII | Total |
|-------------------------------|----|-----|------|----|-----|------|----|-----|------|-------|
| <i>Pasipha</i> sp. 1          | 2  | 2   | -    | 4  | 5   | -    | -  | 1   | 1    | 15    |
| <i>Notogynaphallia</i> sp.    | -  | 1   | 1    | -  | 1   | 1    | -  | -   | 2    | 6     |
| <i>Pasipha</i> sp. 2          | 1  | 1   | 1    | 1  | -   | -    | -  | -   | -    | 4     |
| <i>Pseudogeoplana</i> sp.27   | -  | 4   | -    | -  | -   | -    | -  | -   | -    | 4     |
| <i>Geoplana rubidolineata</i> | -  | -   | 3    | -  | -   | -    | -  | -   | -    | 3     |
| <i>Choeradoplana</i> sp.      | -  | -   | 3    | -  | -   | -    | -  | -   | -    | 3     |
| <i>Pasipha</i> sp.3           | -  | 1   | -    | -  | -   | -    | -  | -   | 2    | 3     |
| <i>Geoplana</i> sp.1          | -  | -   | -    | -  | -   | -    | 2  | 1   | -    | 3     |
| <i>Pseudogeoplana</i> sp.28   | -  | -   | -    | 1  | 2   | -    | -  | -   | -    | 3     |
| <i>Pasipha</i> sp.4           | -  | 1   | -    | -  | -   | -    | 1  | -   | -    | 2     |
| <i>Pseudogeoplana</i> sp.29   | -  | -   | -    | 1  | 1   | -    | -  | -   | -    | 2     |
| <i>Rhynchodemus</i> sp.       | -  | 1   | -    | 1  | -   | -    | -  | -   | -    | 2     |
| <i>Geoplana</i> sp. 2         | -  | 1   | -    | -  | -   | -    | -  | -   | -    | 1     |
| <i>Geoplana</i> sp. 3         | -  | 1   | -    | -  | -   | -    | -  | -   | -    | 1     |
| <i>Geoplana</i> sp. 4         | 1  | -   | -    | -  | -   | -    | -  | -   | -    | 1     |
| <i>Geoplana</i> sp. 5         | -  | -   | -    | -  | -   | -    | -  | 1   | -    | 1     |
| <i>Geoplana</i> sp. 6         | 1  | -   | -    | -  | -   | -    | -  | -   | -    | 1     |
| <i>Geoplana</i> sp. 7         | 1  | -   | -    | -  | -   | -    | -  | -   | -    | 1     |
| <i>Pseudogeoplana</i> sp.30   | -  | -   | -    | 1  | -   | -    | -  | -   | -    | 1     |
| <i>Pseudogeoplana</i> sp.31   | -  | -   | -    | 1  | -   | -    | -  | -   | -    | 1     |
| <i>Pseudogeoplana</i> sp.32   | 1  | -   | -    | -  | -   | -    | -  | -   | -    | 1     |
| <i>Pseudogeoplana</i> sp.33   | -  | 1   | -    | -  | -   | -    | -  | -   | -    | 1     |
| Unidentified specimens        | 1  | 1   | 2    | 1  | -   | -    | 1  | 1   | -    | 7     |
| Richness                      | 6  | 10  | 4    | 7  | 4   | 1    | 2  | 3   | 3    | 22    |
| Abundance                     | 8  | 15  | 10   | 11 | 9   | 1    | 4  | 4   | 5    | 67    |

To describe the community spatial pattern, we used Cluster analysis (Ward's linkage) for the presence and absence of species. To test the relationship between abundance and both air and soil humidity, and organic matter contents, we used stepwise multiple regression analysis. Both analyses were performed employing Systat 11.0.

## RESULTS

Sixty-seven specimens were found, 60 of which were identifiable, belonging to 22 species and six genera of the two subfamilies Geoplaninae and Rhynchodeminae (Table 1).

Overall abundance was higher in areas with primary vegetation, less in secondary vegetation and still less in those with jaboricaba trees (Tables 1 and 2), although differences between the three were not significant (ANOVA,  $F_{(2,6)}=1.201$ ;  $p>0.05$ ). The most abundant species were *Pasipha* sp. 1 and *Notogynaphallia* sp., registered in the three vegetation types. Many species (approx. 60% of the total) were represented by less than three individuals, and 10 were even singletons. Sixteen species, or approx. 73% of all those recorded, were unique, occurring exclusively in only one of the three vegetation types. Most of these were scarce and

TABLE 2

Land flatworm community-structure attributes in areas with jaboricaba trees, areas with deciduous forest (primary vegetation), and areas with regenerating deciduous forest (secondary vegetation) in the Turvo State Park, southern Brazil.

| Attributes       | Primary vegetation | Secondary vegetation | Areas with jaboricaba-trees |
|------------------|--------------------|----------------------|-----------------------------|
| Abundance        | 33                 | 21                   | 13                          |
| Species richness | 18                 | 8                    | 6                           |
| Diversity        | 1.127              | 0.740                | 0.676                       |
| Evenness         | 0.936              | 0.819                | 0.960                       |
| Dominance        | 13.793             | 45.000               | 27.273                      |

represented by only one to three individuals. One unique (*Pseudogeoplana* sp.27), represented by 4 individuals, was moderately abundant (Table 1).

Observed species richness followed the same quantitative sequence as overall abundance, viz. first primary vegetation, then secondary vegetation and finally areas with jaboricaba trees (Tables 1 and 2), although differences between the three types were not significant (ANOVA,  $F_{(2,6)}=1.513$ ;  $p>0.05$ ). The collectors curve did not reach the asymptote (Fig. 2), as estimated species richness for the park was 33. Furthermore, on analyzing seasonal distribution of species richness, no significant differences were found between seasons (ANOVA,  $F_{(2,6)}=2.756$ ;  $p>0.05$ ).

Shannon-Wiener diversity was higher in areas with caducifolious forest than in those with jaboricaba trees (Fig. 3, Table 2), differences only being marginally significant (ANOVA,  $F_{(2,6)}=3.938$ ;  $p=0.08$ ). Regarding evenness and dominance, there were no significant differences be-

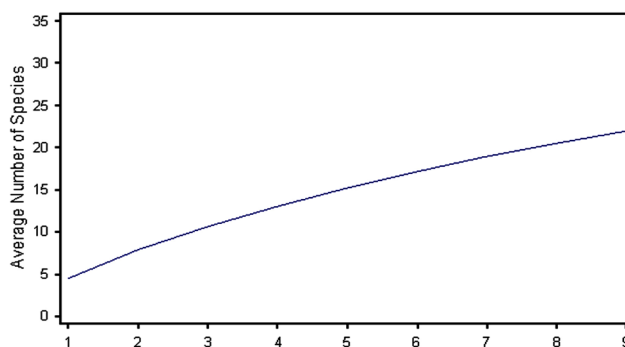


Fig. 2. – Collectors curve after eight samplings in Turvo State Park, southern Brazil.

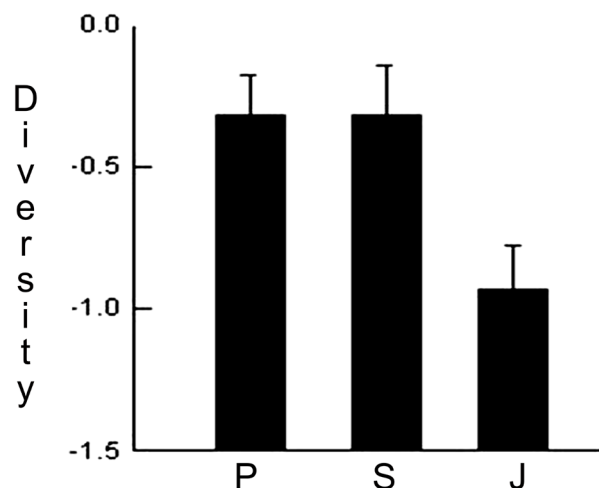


Fig. 3. – Land flatworm diversity in Turvo State Park, southern Brazil. J: areas with jaboricaba trees; P: areas with deciduous forest (primary vegetation); S: areas with regenerating deciduous forest (secondary vegetation).

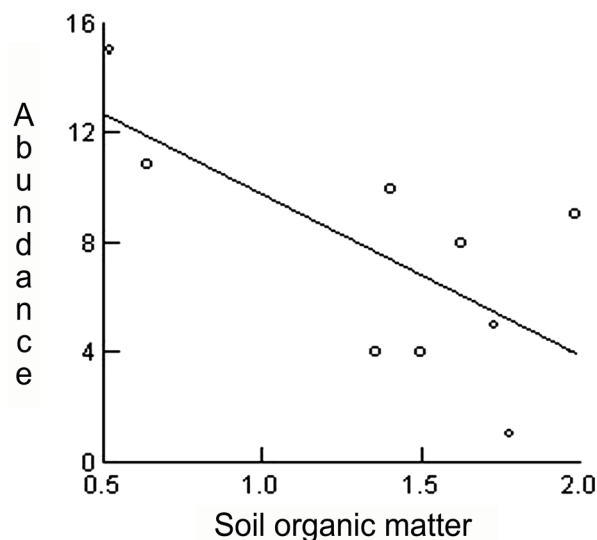


Fig. 4. – Relationship between land planarian abundance and soil organic matter content in Turvo State Park, southern Brazil.



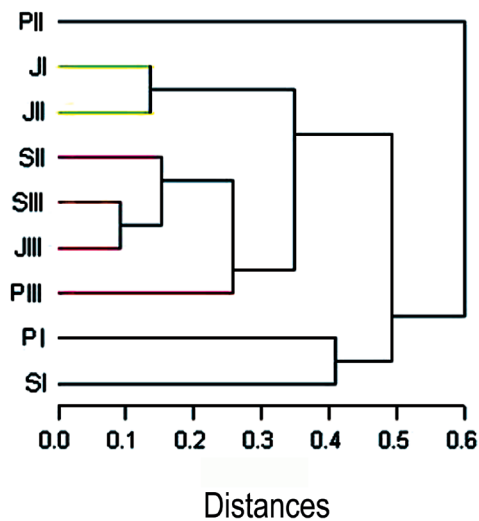


Fig. 5. – Similarity between areas with jaboticaba trees (JI-III), areas with deciduous forest (primary vegetation) (PI-III), and areas with regenerating deciduous forest (secondary vegetation) (SI-III) in Turvo State Park, southern Brazil, based on the presence and absence of land flatworm species analyzed by Cluster.

tween the three vegetation types (ANOVA,  $F_{(2,6)}=0.595$  and 2.665, respectively,  $p>0.05$ ).

The abundance of land flatworms was negatively related to soil organic matter content ( $F_{(1,7)}=5.856$ ;  $r=-0.67$ ;  $p<0.05$ , Fig. 4). There was no statistically significant relationship between abundance and soil humidity ( $F_{(1,7)}=0.498$ ;  $p>0.05$ ) or between abundance and air humidity ( $F_{(1,7)}=0.104$ ;  $p>0.05$ ).

Regarding flatworm community composition, Cluster analysis demonstrated that there was high similarity between sites of distinct vegetation types (Fig. 5). Thus, flatworm communities of the different types of vegetation were not clearly distinct.

## DISCUSSION

The results indicate that Turvo State Park offers refuge to a rich land planarian fauna, presenting a species richness similar to that observed in areas originally covered by araucaria forest in the northeastern part of southern Brazil (CARBAYO et al., 2002). Furthermore, species richness is higher than that observed in Araucaria and Atlantic Rain Forest areas in the National Park of Aparados da Serra (FICK et al., 2003, 2006), or in areas of semi-deciduous forest (ANTUNES et al., 2008) located in the northeastern part of the region. Nevertheless, overall flatworm abundance in Turvo State Park is lower than that observed in the Araucaria and Atlantic Rain Forests, despite the high sampling effort in our study. In another study undertaken in relatively small remnants of deciduous forest located in the central portion of the region (CASTRO & LEAL-ZANCHET, 2005), overall abundance, as well as observed species richness were still lower, in comparison to those obtained in the present study.

Comparative analysis of the three vegetation types shows that abundance and species richness are lower in one of the sites with primary vegetation (PIII), which may be related to its proximity to the Uruguay River. The results obtained for PIII may have been negatively affected by the illegal entrance of hunters, as well as the adverse impact of tourism, which are both more intense in this part of the Park (SEMA/DEFAP, 2005). In general, flatworm diversity was not lower in areas of secondary vegetation located at the southernmost borders of the Park, and surrounded by farms, when compared to lesser disturbed areas of primary vegetation. In contrast, in areas with jaboticaba trees, which are immersed in a matrix of deciduous forest, there was the lowest value for flatworm diversity. The large number of jaboticaba trees, with their profuse fruit attracting large mammals and consequential soil compaction, could possibly negatively affect land flatworm occurrence.

Knowledge on ecological factors affecting land flatworm occurrence or abundance is extremely poor and is restricted to certain invading species of economic significance. Vegetation cover, temperature, air and soil humidity, soil texture, soil pH, soil compaction, and the presence of surface refuges may potentially constitute important factors affecting flatworm occurrence and/or abundance (SPRINGETT, 1976; ALFORD et al., 1998; BOAG et al., 1998a, b; SLUYS, 1998; WINSOR, 1998; CARBAYO et al., 2002; FICK et al., 2006). Intra-specific competition for food is probably the most relevant factor restricting land flatworm population size (BOAG et al. 1998a), by constituting an important factor affecting both their spread and establishment. The present results indicate an additional factor, i.e. soil organic matter content, affecting flatworm abundance. It mainly derives from organic residues of dead animals, litter, dead roots and sloughed off plant cells, as well as exudates from living roots (BROWN & DOUBE, 2004). In our study, soil organic matter content was abundant at sites with distinct vegetation types, thereby negatively affecting flatworm abundance.

Despite the existence of a certain number of scattered exotic species in the park area (SEMA/DEFAP, 2005), the original vegetation has been well-preserved. This might be an explanation for the absence of a clear separation of the flatworm communities of the three main types of vegetation in the study area. Thus, all three types of vegetation are probably offering good general conditions for various flatworm species as a consequence of the degree of preservation of the original vegetation. Similar results were found by LEAL-ZANCHET et al. (2006) when areas planted with *Araucaria angustifolia*, a native species, were compared to those with ombrophilous forest. However, when areas with exotic plant species were compared to those with ombrophilous forest, land flatworm communities were clearly distinct (CARBAYO et al., 2002, LEAL-ZANCHET et al., 2006). The same was found about Araucaria and Atlantic forest (FICK et al., 2006).

The Turvo State Park harbours unique habitats as well as many invertebrate and vertebrate species (GUADAGNIN & ZANINI, 2000; SEMA/DEFAP, 2005). It represents the last large

protected area of deciduous forest immersed in a matrix of cultivated land in southern Brazil. Our results reinforce the need for preserving this natural park as an integral conservation unit.

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