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A New Genus and Species of Euptychiina (Lepidoptera: Nymphalidae: Satyrinae) from Southern Brazil

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Abstract

This paper describes a new genus and a new species of Euptychiina from open grassland habitats (*campos de cima da serra*) in southern Brazil. The systematic position of this new taxon is discussed based on morphological and molecular data, and it is considered sister to *Taydebis* Freitas. Since the *campos* vegetation is considered endangered due to anthropogenic activities, this butterfly species deserves attention and should be included in future conservation plans for this biome.

Introduction

Satyrinae is the most species-rich Nymphalidae subfamily, with more than 2400 species occurring on all continents except Antarctica (Ackery *et al* 1998). Compared to the remaining Nymphalidae, Satyrinae remains poorly understood, with many undescribed genera and species, and with the higher classification still not completely elucidated (Freitas & Peña 2006, Peña *et al* 2006). This is especially critical in the Neotropical region, where more than half of the species of Satyrinae occur, and where most of the new taxa have been described in recent years (Lamas 2004). In the last decade, several new species of Satyrinae have been discovered in the Atlantic Forest, including well-collected localities (Freitas *et al* 2010). However, less than half of these new species are described so far (Freitas 2004, 2007, Freitas *et al* 2010).

The Atlantic Forest is a large heterogeneous biome originally extending more than 3300 km along the eastern Brazilian coast and covering more than 1.1 million km^2 (Morellato & Haddad 2000, Oliveira-Filho & Fontes 2000). The butterfly diversity is not evenly distributed, with species richness concentrated in the "core region" of this biome, in the complex mixed landscapes from São Paulo to Espírito Santo coastal areas, with diversity of almost all groups dropping sharply towards north and south (Brown & Freitas 2000a,b). Nevertheless, the colder habitats in southern Brazil are not just a subsample of the core region of the Atlantic Forest, harboring several endemic species or subspecies in almost all butterfly groups, being especially representative for some groups of Hesperiidae and Lycaenidae (Brown & Freitas 2000b, Iserhard & Romanowski 2004, Grazia *et al* 2008, Romanowski *et al* 2009).

In southern Brazil, the Atlantic Forest *sensu lato* is a mosaic of grassland, shrubland and different forest types (Leite & Klein 1990), including seasonal deciduous

forests in the interior, and the Araucaria Moist Forest, dominated by the Brazilian pine *Araucaria angustifolia* (Bertol.) Kuntze in the upper stratum (Oliveira-Filho & Fontes 2000). In the basaltic highlands of south Brazil, the Araucaria forest form mosaics interspersed with natural grasslands (Fig 1), with the area of the latter increasing towards the south (Behling & Pillar 2006, Overbeck *et al* 2007). This formation is known locally as *campos de cima da serra* (or *campos de altitude*), and most of the highland grasslands have suffered intensive livestock grazing and burning in recent years, and are considered endangered (Overbeck *et al* 2007).

In the last 14 years, the research project "Borboletas do Rio Grande do Sul" intensively sampled many types of habitats belonging mainly to the Atlantic Forest in the state of Rio Grande do Sul, including the basaltic highlands in the northeast region of the State (Iserhard & Romanowski 2004, Grazia *et al* 2008, Romanowski *et al* 2009, Iserhard *et al* 2010). These efforts uncovered a butterfly diversity much higher than previously known. Besides several new records for the state, intensive fieldwork resulted in the capture of an undescribed highland species described here. Many additional individuals were also found in five collections, all of which are used as type material. Accordingly, this study describes a new genus and species of Satyrinae, and discusses its systematic position within the tribe Euptychiina.

Fig 1 General view of the habitat of *Prenda clarissa*. a) Contact of open field with the *Araucaria* forest; b) General view of a grassland area.

Material and Methods

Dissections were made using standard techniques, where legs, palpi, and abdomens were soaked in hot 10% KOH solution for 10 min before dissection, and dissected parts were stored in glycerol. Taxonomic nomenclature follows Lamas (2004), modified after Peña *et al* (2006) and Wahlberg *et al* (2009).

The acronyms for the collections are: BMNH, British Museum (Natural History), London, England; CGCM, Coleção Carlos Guilherme Costa Mielke, Curitiba, Paraná, Brazil; CLDZ, Coleção de Lepidoptera, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil; DZUP, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; MC, Coleção Moser, São Leopoldo, Rio Grande do Sul, Brazil; MECB, Museu Entomológico Ceslau Biezanko, Departamento de Fitossanidade, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil; OM, Coleção Olaf Mielke, Curitiba, Paraná, Brazil; ZUEC, Museu de Zoologia da Universidade Estadual de Campinas, Unicamp, Campinas, São Paulo, Brazil.

DNA sequences of cytochrome c oxidase subunit I (cox1), elongation factor-1 alpha (ef-1alpha) and wingless (wgl) were sequenced for one individual (DNA voucher BR-AVLF-1) (ca. 3127 bp total), as described in Silva-Brandão *et al* (2008). These sequences were analyzed together with the dataset compiled by Peña *et al* (2010), using Bayesian Inference. Bayesian analyses (Huelsenbeck *et al* 2001, 2002) were carried out for the combined data set under the model GTR + Γ, using the program MrBayes 3.2 (Ronquist & Huelsenbeck 2003). Analysis of combined data by Bayesian methods permits partition-specific substitution models and parameters (Nylander *et al* 2004). For that reason, all substitution model parameters (gamma shape, character state frequencies, substitution rates of GTR model) were allowed to vary across partitions (= genes). Fifteen simultaneous chains were conducted for 20.0 x10^6 generations, sampling trees every 1,000 cycles. The first 10,000 trees were discarded as "burn in".

Prenda Freitas & Mielke, new genus (Figs 2, 3, 4)

Type species: Prenda clarissa *Freitas & Mielke, new species*

Adult diagnosis. Eyes with sparse hair, entirely dark brown. Palpus length almost 2.0 times head height, covered abundantly with long brown and beige long scales; first segment about one-third of the second,

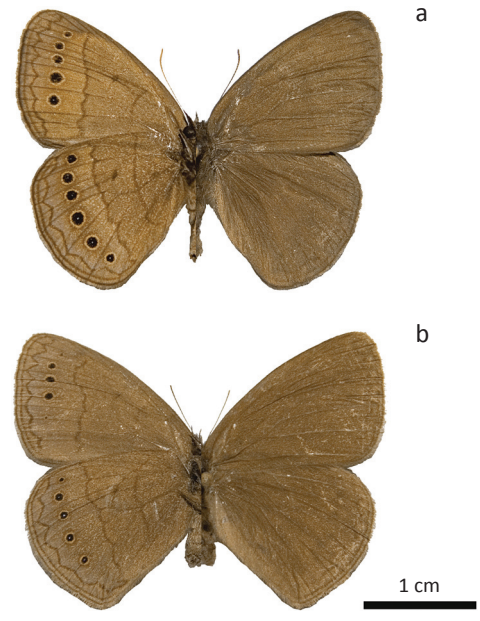

Fig 2 *Prenda clarissa*, ventral on the left, dorsal on the right. a) holotype male from Bom Jesus, Rio Grande do Sul, Brazil; b) allotype female, from Cambará do Sul, Rio Grande do Sul, Brazil.

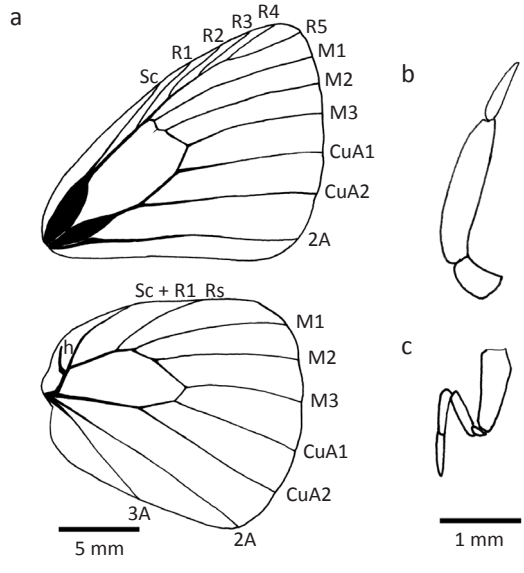

Fig 3 *Prenda clarissa*. a) male wing venation; b) male palpus; c) male foreleg.

second about twice the length of the third (Fig 3b). Antenna of males 7.0-9.0 mm in length, with 32-34 segments extending to mid-costa; shaft dark orange, dorsally covered by dark brown scales, club with 11-12 segments, not conspicuously developed. Both wings rounded in males and females; male wing venation very similar to *Taydebis* Freitas (2003), especially by the distal edge of the forewing discal cell, where the crossveins form a nearly straight line from the radial stem to M_3 (Fig 3a). Male foreleg covered by long dark brown and beige

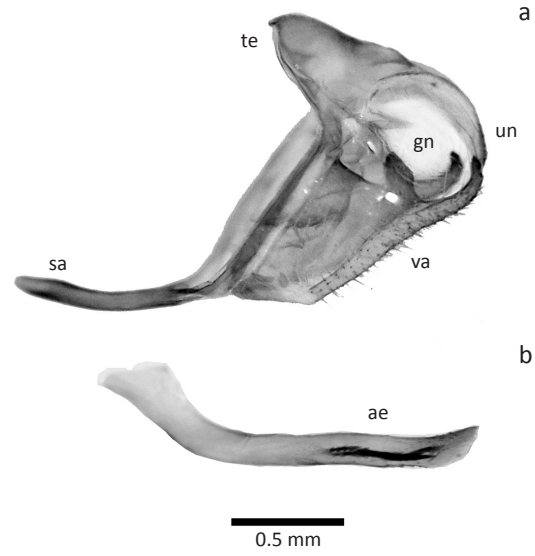

Fig 4 Male genitalia of *Prenda clarissa*. a) lateral view; b) aedeagus; sa = saccus, va = valva, ae = aedeagus, un = uncus, te = tegumen, gn = gnathos.

long scales and with one tarsomere as long as tibia (Fig 3c). Male genitalia presented in Fig 4. Adults similar to *Taydebis*, but easily distinguished from the later by their smaller size and general wing pattern in both sexes, and by the male genitalia with uniquely characteristic valva. *Etymology.* The generic name is feminine. The word "prenda" ("moça gaúcha", Nunes & Nunes 2007) designates a young woman from Rio Grande do Sul, one of the States from where type specimens were obtained. Prenda is therefore used here in a figurative sense in honor of this well-regarded folklore figure.

Prenda clarissa *Freitas & Mielke, new species (Figs 2, 3, 4)*

Satyrinae sp. Iserhard *et al* (2010): 312.

Diagnosis. Prenda clarissa is the only known species in the genus, and is easily distinguished from all other sympatric species of Satyrinae by its unique rounded wing shape, and a well developed line of black ocelli on the ventral surface of both wings.

Male (Fig 2a). Body entirely dark brown. Forewing length 17.0-19.5 mm (average 17.7 mm, SD = 0.71, n = 19); hindwing length 13.5-15.5 mm (average 13.9 mm, SD = 0.48, n = 19). Dorsal ground color dark brown with no markings, restricted to weakly visible marginal and submarginal lines in both wings. Ventral ground color of wings same as dorsal; forewing crossed by two dark brown lines, the first concave, extending from discal cell to 2A one third from base; the second wavy, extending from costa to 2A at two-thirds from the wing base and delimiting a lighter distal area; a dark brown zigzag submarginal line and a brown regular marginal line

extending from costa to 2A; five black ocelli outlined by a yellow ring, and with white pupil in spaces R5-M1 (ocellus 1), M1-M2 (2), M2-M3 (3), M3-CuA1 (4) and CuA1-CuA2 (5). Hindwing crossed by two dark brown irregular lines from costa to anal margin, the first one-third from the wing base and the second two-thirds from it; second crossing line delimiting a lighter distal area; a dark brown zigzag submarginal line and a brown regular marginal line extending from costa to 2A; a series of five (or rarely six) black ocelli outlined by an yellow ring, and with white pupil can be found in spaces Rs-M1 (ocellus 1), M1-M2 (2), M2-M3 (3), M3-CuA1 (4), CuA1-CuA2 (5) and CuA2-2A (6). Male genitalia (Fig 4a, b): Saccus long, about half of the total genitalia, narrow in lateral and ventral view; tegumen rounded; appendices angulares extremely reduced and inconspicuous, the valvae connecting directly to the combined ventral arms of the tegument and dorsal arms of the saccus; gnathos pointed, about half the length of the uncus; uncus elongated, curved downwards and pointed; valvae elongated, with a long marginal fold extending from saccular to apical region, ending in a narrow apical process, and with an additional dorsal and bumped process proximal to the apical process, giving an appearance of a bifurcated end; aedeagus straight, with phallobase bent upwards; a long conspicuous cornutus present; fultura inferior (juxta auct.) weakly sclerotized.

Female (Fig 2b). Body entirely dark brown. Forewing length 18.0-20.0 mm (average 18.92 mm; SD = 0.73, n = 7); hindwing length 14-16 mm (average 15.1 mm; SD = 0.89, n = 7). General color and pattern very similar to, but in general paler than that of males and with wings more rounded.

Remarks on color variation. Variation on the dorsal wing surfaces was not observed. In the ventral surface of both wings the number of ocelli varied: forewing ocelli 1, 2 and 5 can be extremely reduced, and 1 and 5 can be absent in some individuals; hindwing ocelli number 1 and 6 can be small or absent in some individuals.

Habitat. Adults were observed only in open habitats and grasslands, flying among grass patches and usually perching on the ground. Based on recent field records, *P. clarissa* seems to be associated with wet areas where the soil is permanently flooded. The species also appears to occur in altitudes above 800 m in the Serra do Mar and Serra Geral Mountain range, except for two records at the lowlands, from Pelotas, and Pinheiro Machado, Rio Grande do Sul.

Distribution. The studied specimens were obtained from 12 highland sites (800-1400 m, a.s.l.) in south Brazil: Bom Jesus (28°38'S, 50°29'W), Cambará do Sul (29°10'S, 50°08'W), São Francisco de Paula (29°25'S, 50°22'W) in Rio Grande do Sul, Bom Jardim da Serra (28°21'S, 49°34'W), Lages (27°47'S, 50°20'W), Santa Cecília (26°56'S, 50°20'W), São Joaquim (28°17'S, 49°55'W), Curitibanos (27º16'S, 50º35'W), and Urupema (27º55'S, 49º51'W) in Santa Catarina, Balsa Nova (São Luiz do Purunã) (25°27'S, 49°42'W), São José dos Pinhais (Pilão de Pedra) (25°27'S, 49°42'W) and Castro (24°47'S, 50°01'W) in Paraná, and from two lowland sites: Pelotas (31°46'S, 52°21'W), at sea level, and Pinheiro Machado (31°35'S, 53°34'W), at 250 m, both in Rio Grande do Sul. All individuals were collected in open grassland vegetation (the *campos* vegetation).

Remarks. The species is referred as "Satyrinae sp." in Iserhard *et al* (2010).

Etymology. The specific epithet is feminine and refers to *Clarissa*, a main character and also the title of the first novel of Érico Veríssimo (written in 1933), a famous Brazilian writer born in Cruz Alta, Rio Grande do Sul on December 17, 1905.

Holotype. Male (Fig 2a) from Bom Jesus (28º38´S, 50º29´W), 900-1000 m, Rio Grande do Sul, Brazil, collected by A. Moser on February 9, 1996. Deposited in the Museu de Zoologia (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. Labels on the holotype (five labels, separated by transverse bars): BRASIL - RS, Bom Jesus, 900-1000 m, leg. A. Moser, 19.2.96 / 13 / HOLÓTIPO / Holotype *Prenda clarissa* Freitas & Mielke / ZUEC LEP 3221.

Allotype. Female (Fig 2b), from Cambará do Sul (29º10´S, 50º08´W), Rio Grande do Sul, Brazil, collected by A. Moser on April 3, 1994, also deposited in the ZUEC. Labels on the allotype (four labels, separated by transverse bars): Itaimbezinhi [sic] [Itaimbezinho], RS - Brasil, leg. A. Moser, 3.4.94 – 1000 m / ALÓTIPO / Allotype *Prenda clarissa* Freitas & Mielke / ZUEC LEP 3222.

Paratypes. **ZUEC** – *Rio Grande do Sul*: one male (all wings and part of abdomen removed, with both antennae broken, genitalia prepared AVLF 0009) 06-IV-2008, boundaries of Floresta Nacional de São Francisco de Paula, São Francisco de Paula, F. Steiner leg., ZUEC LEP 3223; *Santa Catarina*: one male (without right antenna) 11-III-2001, Santa Cecília, 1200m, A. Moser leg.; *MC – Rio Grande do Sul*: one male and one female, 03-IV-1994, Itaimbezinho, Cambará do Sul, 1000 m, A. Moser leg.; two males, 19-II-1996, Bom Jesus, 900-1100 m, A. Moser leg.; *Santa Catarina*: one male, 11-III-2001, Santa Cecília, 1200 m, A. Moser leg.; one male, 28-XII-2002, Urupema, Morro de Torres, 1700 m, A. Moser leg.; **OM** – *Santa Catarina*: two males 16-XI-2002, Fazenda Santa Rita, 13

km East of Bom Jardim, 1200 m, O. Mielke leg., OM 58.949, OM 58.994; *Paraná*: one male 19-III-1988, São José dos Pinhais, 850 m, O. Mielke leg., OM 16.929; **CGCM** – three males and one female 6-II-1993, Pilão de Pedra, São José dos Pinhais, 850 m, C. Mielke leg., CGCM 24.001, CGCM 24.002, CGCM 24.003, CGCM 24.004; **DZUP** – *Rio Grande do Sul*: one male 23-II-1983, Bom Jesus, O. Mielke & M. Casagrande leg., DZ 16.647; one male and one female 28-II-1980, Itaimbezinho, Cambará do Sul, H. Steiniger leg., ex col. H. Ebert, DZ 16.663, DZ 16.705; one female 7-IV-1968, Pelotas, C. Biezanko leg., ex col. H. Ebert, DZ 16.670; *Santa Catarina*: one male 23-II-1973, Painel, Lages, 1000 m, O. Mielke leg., DZ 16.633; one male 22-II-1973, Campo Alto, Santa Cecília, 1200 m, O. Mielke leg., DZ 16.698; one female 23-II-1983, 14 km south of São Joaquim, O. Mielke & M. Casagrande leg., DZ 16.691; one male no data, São Joaquim, H. Ebert leg., ex col. H. Ebert, DZ 16.684; two males 16-XI-2002, Fazenda Santa Rita, 13 km L Bom Jardim, 1200 m, O. Mielke leg., OM 58.949, OM 58.994; one male, 27-II-1973, Curitibanos, 950 m, H. Ebert leg., ex col. H. Ebert, DZ 20.977; *Paraná*: one male 9-II-1977, one male 18-II-1982, Pilão de Pedra, São José dos Pinhais, 850 m, O. Mielke leg., DZ 16.654, DZ 16.640; one male 19-III-1988, São José dos Pinhais, Paraná, 850 m, O. Mielke leg., OM 16.929; one female 7-VIII-1977, São Luiz do Purunã, Balsa Nova, 900 m, O. Mielke leg., DZ 16.677; one male 25-26-III-2007 São Luiz do Purunã, Balsa Nova, 900 m, Beltrami leg., DZ 20.970; **CLDZ** – *Rio Grande do Sul*: one male 08-III-2008, boundaries of Floresta Nacional de São Francisco de Paula (29º25'26"S, 50º22'04"W), São Francisco de Paula, 900 m, F. Steiner leg. (DNA Voucher BR-AVLF-1); one female 08-III-2008, boundaries of Floresta Nacional de São Francisco de Paula (29º25'26"S, 50º22'04"W), São Francisco de Paula, 900 m, F. Steiner leg; **MECB** – *Rio Grande do Sul*: one female 08-IV-2009, Fazenda São José, Pinheiro Machado, 257m, (31º35'23"S, 53º34'04"W); **BMNH** – *Paraná*: one male I-1913, one male II-1913 (genitalia prepared BMNH(E)#808405, VIAL 8857), two males II-1913, Castro, Paraná, 950 m, E. D. Jones leg.; one female, no data, same locality.

Molecular data. DNA sequences of cytochrome c oxidase subunit I (*cox1*), elongation factor-1 alpha (*ef*-1alpha) and wingless (*wgl*) were sequenced for one individual of *Prenda clarissa* (DNA voucher BR-AVLF-1) (ca. 3127 bp total). GenBank accession numbers: HQ444284, HQ444285 and HQ444286.

Phylogenetic position. Based on the *cox1*, *ef*-1alpha and *wgl* sequences of one freshly collected individual, this taxon emerged as member of the '*Pareuptychia* clade' (*sensu* Peña *et al* 2010), and sister to *Taydebis peculiaris* (Butler, 1874) in a Bayesian inference including 127 taxa (all those on Peña *et al* 2010 plus *Prenda clarissa*), with strong Posterior Probability support (Fig 5). The new taxon, *Prenda clarissa*, share with *T. peculiaris* at least two

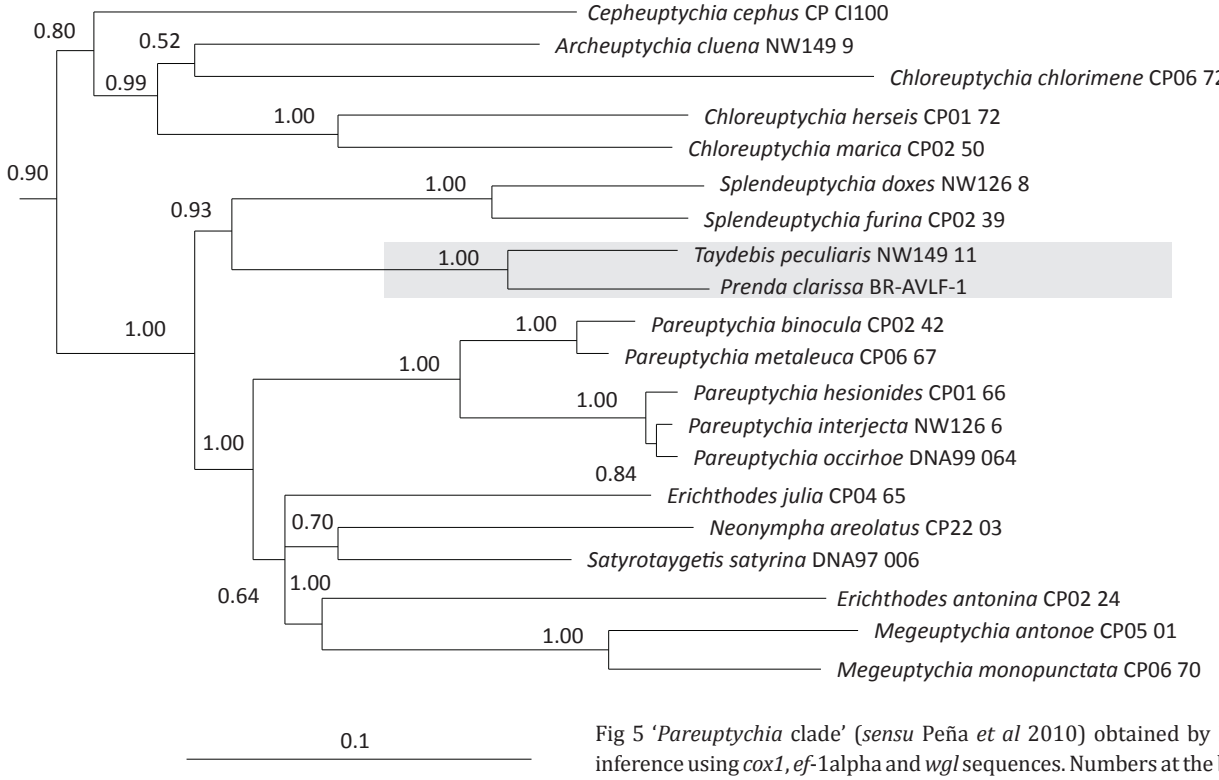

Fig 5 '*Pareuptychia* clade' (*sensu* Peña *et al* 2010) obtained by Bayesian inference using *cox1*, *ef*-1alpha and *wgl* sequences. Numbers at the branches are posterior probability values for the node to the right of the number.

morphological traits: 1) the presence of a cornuti and 2) the "rounded" wing profile (Freitas 2003). However, these two characters are not unique among the Euptychiina, and cannot be used at this moment as good synapomorphies for this clade.

Discussion

Many of the recognized Neotropical Satyrinae genera were erected based on weak morphological characters, including general shape, color, average size and some morphological traits of wide occurrence (see Forster 1964). As a consequence, recent studies have shown that several Euptychiina genera are polyphyletic (Peña *et al* 2006, 2010), and for those that are monophyletic, synapomorphies should be identified and described (see an example for the genus *Moneuptychia* Forster, 1964, in Freitas 2007). The description of the new genus *Prenda* was supported by: 1) the absence of clear unique synapomorphies uniting *P. clarissa* to any other known genus in Euptychiina, and 2) a unique valvar shape, which found no parallel in any known Euptychiina genera.

The systematics of Satyrinae in general, and Euptychiina in particular, need further studies before a clearer picture of the evolutionary relationships of the group as a whole can emerge (Freitas 2007). Even if some recent molecular studies have brought some light to the internal relationships of Satyrinae (Murray & Prowell 2005, Peña *et al* 2006, 2010), the last comprehensive morphological study of the group was that of Miller (1968). Furthermore, most clades identified by recent analyses of DNA sequence data have no recognized morphological synapomorphies. The present paper is a clear example of the consequences of this uncertainty in the subtribe Euptychiina, and even with adequate morphological data of wing venation and genitalia, the placement of the present taxa was only obtained with the aid of molecular data.

Based on the present data, despite the two records on lowland areas, the presence of *P. clarissa* appears to be much linked to the open grasslands habitats in the Mountains in south Brazil, and could be considered characteristic to the *campos de altitude* vegetation. In this case, *P. clarissa* could be as vulnerable as its habitat (Overbeck *et al* 2007, Bond-Buckup 2008), and needs to be included in future conservation plans for this biome.

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