Taxonomy and conservation concerns of the critically endangered *Roppaneura beckeri*, a phytotelm-breeding damselfly in the southern Brazilian Atlantic Forest

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Abstract

Phytotelm-breeding Odonata are rare: from the 6,300 known species of these charismatic freshwater organisms, only a small number of about 50 develop in phytotelmata habitats. Mainly members of the damselflies (suborder Zygoptera) are dependent on this special type of environment. The small coenagrionid *Roppaneura beckeri* Santos (Odonata Coenagrionidae Protoneurinae), a damselfly endemic to the Brazilian Atlantic Forest, is the only known Odonata breeding in the terrestrial umbellifers of *Eryngium floribundum* (Cham. et Schltdl.). This is a species-specific association with a hostplant unparalleled in the order. It also is the only species within the subfamily Protoneurinae to occupy phytotelmata habitats. Here, we report on a population of *R. beckeri* rediscovered after 42 years and recorded for the first time from the southern Atlantic Forest from the state of Paraná. The morphology and the distribution of this species is reviewed and based on these primary data future conservation strategies are discussed. We suggest including *R. beckeri* as a priority species for dragonfly conservation policies due to its exclusive biological characteristics, evolutionary relevance, and occurrence in urban to peri-urban landscapes.

Key words: conservation, Plateau Paranaense, Zygoptera, Coenagrionidae, urban insects.

Introduction

The aquatic insects of the order Odonata, known as dragonflies (suborder Anisoptera) and damselflies (suborder Zygoptera), have successfully colonized all sorts of freshwater environments. Their pre-imaginal stages develop in big rivers, streams, pools, swamps, bogs and small water bodies found in or upon plants, the last including phytotelmata (Corbet, 1999). From the globally known ca. 6,300 species of Odonata (Paulson and Schorr, 2020), only a small group of at least 50 species within 24 genera from both suborders are known to inhabit phytotelmata (Corbet, 1999; updated). The phytotelm-breeding Odonata are found in Neotropical, Oriental, Pacific, Afrotropical, and Australasian regions, however they are significantly more diverse in the neotropics (Corbet, 1983). Some species are obligatory phytotelm-breeding (dendrolimnetobiont), thus evolved to occupy this strong specific habitat, while others are facultative (dendrolimnetophiles or dendrolimnetoxenes), as a result of their larvae eventually being found in this kind of mesohabitat (Corbet, 1983). Zygoptera has the highest number of species among phytotelm-breeding Odonata, which includes at least 37 species from Argiolestidae and Coenagrionidae (Kitching, 2000), however this number may be much higher pending to be updated. For instance, in Brazil there are currently 36 species of the mandatory phytotelm-breeding species of the subfamily Pseudostigmatinae of Coenagrionidae (Pinto, 2020).

Roppaneura beckeri Santos (Odonata Coenagrionidae Protoneurinae) is a small damselfly endemic to Brazil, and the only known odonate with larval development associated with terrestrial umbellifers of the genus *Eryngium* (Campos, 2010). It also is the sole species among

the protoneurid lineages (Coenagrionidae Protoneurinae) to occupy phytotelmata habitats, breeding exclusively on the *Eryngium floribundum* (Cham. et Schltdl.) as a hostplant. This unique association with phytotelmata habitats in the group is intriguing from an evolutionary standpoint considering the species belongs to a clade of largely semi-lotic to lotic dwelling species (Pimenta *et al.*, 2019).

The first specimens of the monotypic genus Roppaneura were collected between 1963 and 1964 from two localities in the Atlantic Forest domain at the Poços de Caldas municipality in the state of Minas Gerais, Brazil (Santos, 1966). Another population was discovered about 300 km from the type locality also in Minas Gerais with specimens collected between 1976 and 1978 (Machado, 1981a). The biology of this population was broadly studied by the late Angelo B. M. Machado (Machado, 1976; 1977; 1981a; 1981b). He did several pioneering observations in the field based on surveys with adults, 350 larvae, analyses of 70 plants and experiments with larvae about the use of resources and how to avoid cannibalism. Machado (1981a; 1981b) observed that populations of different ages could live together and concluded the species is semivoltine taking up 13 stadia to adult's emergence. Moreover, he also reported characteristics of oviposition, occupation in the plant according to the larval stadium, territorialism (at least in larvae with advanced development) and cannibalism behaviour of the larvae and aspects of adult's emergence (Machado, 1981a; 1981b). Although available only as abstracts from scientific meetings, Machado's extraordinary findings have been largely cited in the essential reviews on the biology of Odonata (Corbet, 1983; 1999). Since after these seminal studies as far as we know no further investigation was carried out on these sites, thus the conservation status of these populations of damselflies, as well as their habitat integrity, are unknown. Many of the known specimens of *R. beckeri*, including its holotype, were deposited in the entomological collection of the Museu Nacional of Federal University of Rio de Janeiro lost in the fire of 2018 (Kury *et al.*, 2018).

Records of R. beckeri are from a few localities within the Atlantic Forest in Minas Gerais, a state with high levels of deforestation within the Atlantic Forest between 2016 and 2017 (Fundação SOS Mata Atlântica and INPE, 2018). Based on the published data (Machado, 1981), it has been more than 40 years since the last specimens of R. beckeri were collected at the end of the 1970's decade, and it was considered a Critically Endangered species for the IUCN Red List of Threatened Species (Pinto et al., 2020). Unexpectedly, a population of this damselfly was recently discovered at a small patch of vegetation in an urban area in southern Brazil and data on this population appeared earlier on the literature (Pimenta et al., 2019) based on material in this study which is now available in full details herein. Knowledge about the distribution of this species is essential in order to plan conservation actions, avoid mistakes on the knowledge of evolutionary patterns and to plan further studies.

The goal of this study is to comprehensively record the first occurrence of *R. beckeri* in the southern Atlantic Forest from Paraná State. In addition, the morphology and distribution of this species are reviewed, aiming to provide primary data for the elaboration of future strategies of conservation.

Materials and methods

Specimens of *R. beckeri* were collected between 2017 and 2020, from a small swampy area (approximately 50 square meters) covered with "gravatás" (*Eryngium* spp.), at Centro Politécnico Campus of the Federal University of Paraná (UFPR), Curitiba, Paraná, Brazil. This spot is in the transition of a small wood patch and a lawn (25°27'11"S 49°14'06"W, 914 m a.s.l.). The metropolitan area of Curitiba is located within the Atlantic Forest (IBGE, 2019) in the southern limit of the domain, the main phytogeographic unit is the steppe (grasslands) with scattered forested areas (Roderjan *et al.*, 2002). The climate is humid subtropical, with 1,515 mm of annual mean precipitation, 12.9 °C of mean minimum and 22.5 °C mean maximum temperatures (Mendonça and Danni-Oliveira, 2007).

Adults, larvae from the water accumulated in the leaves of *E. floribundum* and one exuviae were collected. Adults were fixed in acetone and larvae reared to emergence in the laboratory following modified versions from the procedures detailed in Paulson (2009) and Carvalho (2007), respectively. Collections were undertaken under the licenses ICMBio/SISBio 58681-1 and 58681-2. All specimens are deposited at the Entomological Collection "Pe Jesus Santiago Moure", Department of Zoology, Federal University of Paraná, Curitiba, Brazil (DZUP).

Terminology, abbreviations, and general procedures follow Pinto and Kompier (2018). We followed Corbet (2002) for the postembryonic development terminology

of Odonata, consequently we applied the term larva instead of nymph, such as recently reinforced by Sahlén et al. (2016). Anatomical structures were dissected, cleaned, and sometimes retreated with acetone depending on photographic technique. Specimens were analysed with the aid of a stereomicroscope. Determination and revision of the morphology were undertaken by comparison with the original description (Santos, 1966) and additional data on Garrison et al. (2010) and photos from the type series. A voucher specimen of the phytotelmata plant was determined as E. floribundum using Cardoso's (2017) key and was deposited at Herbarium of the Department of Botany, Federal University of Paraná (UPCB). Images of external morphology were obtained with a Scanning Electron Microscope TESCAN VEGA3 LMU and stereomicroscope LEICA MZ16, equipped with a camera LEICA DFC 500, and the source images arranged with auto-montage software LAS MONTAGE (Version 4.7).

The geographic data was obtained from the literature and voucher material. A continuous search was undertaken in several online databases (Web of Science, SciELO, etc.) with the keywords *Roppaneura*, beckeri and Protoneuridae, alone or combined. Localities lacking coordinates were georeferenced using IBGE (2011) and checked in Google Earth Pro (Version 7.3.3.7786). The map was executed with the Open Source Geospatial Foundation Project, QGIS Geographic Information System (2019, http://qgis.osgeo.org).

Results

The population of this remarkable damselfly was found at the Centro Politécnico Campus, Curitiba municipality, state of Paraná (figures 1, 2a-b). All previously known records are also within the Atlantic Forest. Its known records are in the northern portions of the Parana Forest biogeographical province, two at the Pocos de Caldas and one at Florestal municipalities, all in the state of Minas Gerais (figure 1). The new locality is about 500 km from the nearest northern record (Poços de Caldas) and is the first record from within the Araucaria Forest province and for the state of Paraná (figure 1). A total of 14 specimens were collected in an urban site with high human activities (figures 2a-b). The sampling site (figure 2c) has an assemblage of plants with at least two species of Eryngium, where individuals of E. floribundum are predominant (figure 2d). Larvae were found only in the waterfilled axil of E. floribundum (figure 2e), while adults were observed at the same patch exhibiting numerous behaviours, including territorialism and mating (figure 2f).

Through comparative morphology analyses it was possible to see variations in the coloration of the body. The major colour variations are in the size and shape of the dark areas. Specimens from Curitiba are conspicuously darker than the previously reported populations from Minas Gerais (figure 3). Surprisingly, adults present the same larger dark areas already a few hours after emergence, and a few days later, a bluish-white pruinosity appears on S8-10. Moreover, an updated catalogue of the species and SEM images of morphological characters are also made available (figure 4).

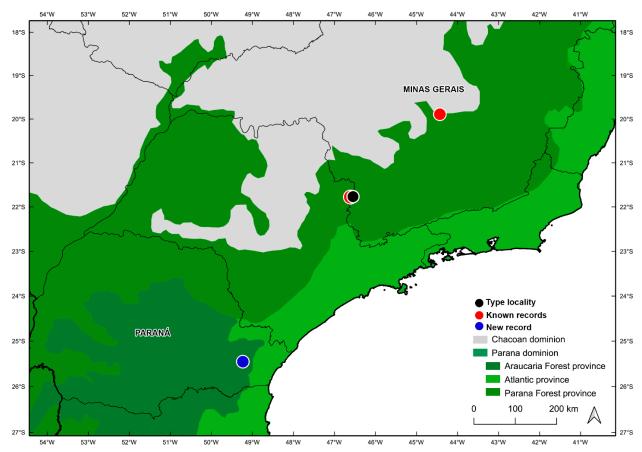


Figure 1. Map of south eastern Brazil with occurrence records of *R. beckeri*. Blue symbol represents the new record, black symbol represents type locality and red symbols represent previously known records. Biogeographical regionalization based on Morrone (2014).

Roppaneura beckeri Santos 1966 ZOOBANKLSID:

http://zoobank.org/urn:lsid:zoobank.org:act:2AF2ECEA-5038-4220-AC71-23E577BFAB36 (figures 1, 2e, 2f, 3a-f, 4a-f)

Roppaneura beckeri Santos, 1966: 1-4, figs 1-11 (description of male holotype and allotype, BRAZIL, Minas Gerais State, Poços de Caldas municipality: Morro [do] Ferro, illustrations of Hw of holotype and allotype, prothorax, head and caudal appendages in dorsal view, thorax in lateral view, cercus in dorsolateral and lateral views, genital ligula in lateral view of the holotype, biological notes); —Machado (1976: 895-896, ecological and biological notes); —Machado (1977a: 10, biological notes); —Machado (1977b: 11, ecological and behavioral notes); —Rowe (1980: 285, mention): —Machado (1981a: 41-42, life history. ecological and biological notes); -Machado (1981b: 149-150, ecological and larval behavior notes); -Baker (1983: 206, mention); bet (1983: 33, 35, 37-39, 40-45, 47, fig 2, biological notes); —Westfall (1984: 3, mention); —Davies and Tobin (1984: 117, catalog); —Williams (1987: 130, mention); —Santos (1988: 274, 334, catalog); —Machado and Costa (1990: 301, mention); —Garrison (1991, mention [updated version Garrison and von Ellenrieder 2019: 23]); -Watson (1992: 197, mention); —Utzeri (1992: 4, mention); —Bridges (1994: iii. 46, iv. 7, v. 5, vi.1, vii. 29, ix. 19, x. 40, xiii. 52, figs. 442,catalog); -Steinmann (1997: 455, catalog); —Moore (1997: 16, notes on conservation); —Santos (1988: 334, mention); —Corbet (1999: 10, 17, 26, 82, 138, 144-145, 163, 221, 617, 622, figs. 5.10, biological notes); -Costa et al. (2000: 7, mention); -Kitching (2000: 316, 319, biological notes); —Silsby (2001: 122, biological notes); —Costa et al. (2004: 15, mention); —Lencioni (2005: 227, fig. 153, reproduction of illustrations from Santos 1966); -- Corbet et al. (2006: 36, mention); -- Lencioni (2006: 321, fig. F28, mention); —Heckman (2008: 577, 579, fig. 3.1.689, key, reproduction of illustrations from Santos 1966); —Pessacq (2008: 511, 514, 518-522, 526, fig. 2, 6-9, phylogenetic analysis); -von Ellenrieder and Garrison (2009: 103, fig. 53, key); —Campos (2010: 325, biological notes); —Garrison et al. (2010: 341, 346, 383-385, figs. 2529-2537, distribution map, illustrations of head pronotum

and mesostigmal plates in dorsal view, pronotum in dorsolateral view and S8-10 in lateral view of the female, wings, genital ligula in lateral and ectal views, S10 in lateral and dorsolateral views of the male); —Pessacq *et al.* (2012: 71, mention); —Neiss and Hamada (2012: 31, 35-37, 40, fig. 3-4, description of F-0 larvae, photos of F-0 habitus, head and prementum in dorsal view, left antenna, labial palp, mandibles; male gonapophyses in ventral and lateral views, female last abdominal segments, median and lateral gills in lateral view of exuvia); —Neiss and Hamada (2014: 253, fig. 122, key); —Hämäläinen (2015: 21, 131, eponym catalog); —Pinto (2016: 14, mention); —Lencioni (2017: 235, 257, 238, 243, fig. 129–130, male and female habitus); —Lozano *et al.* (2018: 475-476, 483, fig. 14.6.25., key); —Pimenta *et al.* (2019: 399, 402-403, figs. 1, 3-4, phylogenetic analysis); —Pinto and Araujo (2020: 108, 120-121, discussion of relationship with the genus *Forcepsioneura* Lencioni 1999).

Material examined (9 males, 5 females)

BRAZIL. Paraná State: 1 female, Curitiba municipality, Centro Politécnico Campus - UFPR, edge of the wood in the drainage swampy area of the football fields of the Educação Física, with *Eryngium* sp. (Apiaceae) (25°27'11"S 49°14'06"W, 914 m a.s.l.), 13.XII.2017, A.P. Pinto leg. (DZUP 500079; DNA-RO 001); 3 males, 2 females, same data but 14.XII.2017 (DZUP 5000080-500084); 1 male larva, same data but collected in *Eryngium floribundum*, 11.X.2019 (emerged 03.I.2020), (DZUP 500350); 1 male larva, same data but emerged 14.X.2019 (DZUP 500351); 2 males, same data but 18.XI.2019, M.C. Alencastro leg. (DZUP 500352-500353); 2 males, 2 females, same data but 12.XII.2019, A.P. Pinto and L. Polizeli leg. (DZUP 500354-500357).

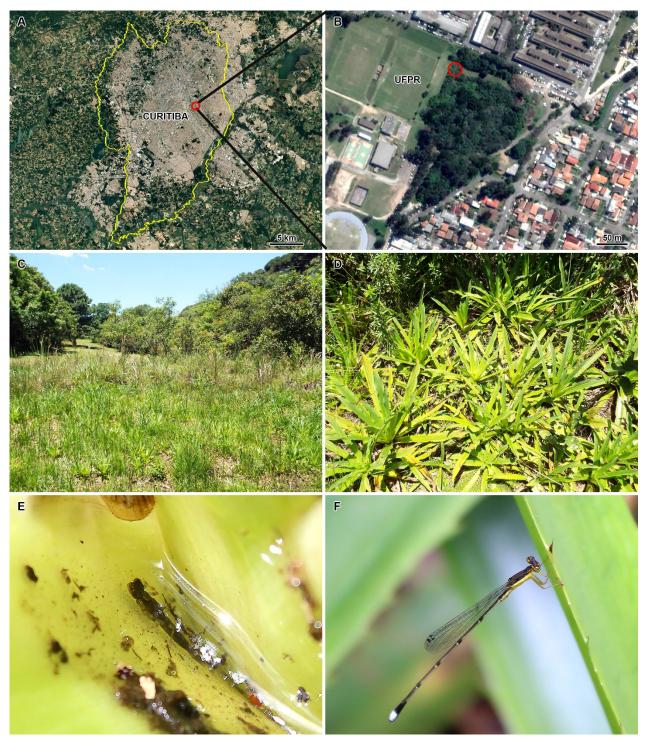


Figure 2. Sampling site of larvae and adults of *R. beckeri* at Curitiba municipality, Paraná state, Brazil: **(a)** Curitiba municipality (yellow outline); **(b)** small patch of vegetation at Centro Politécnico Campus where the population of *R. beckeri* was discovered; **(c)** overview of sampling site; **(d)** group of *E. floribundum*; **(e)** larva at the water-filled axil of *E. floribundum*; **(f)** adult on leave of *E. floribundum*. Sampling site = red circle. Abbreviation UFPR = Federal University of Paraná, Centro Politécnico Campus.

Type repository

Holotype male by original designation and allotype female in MNRJ, examined by photos (©2018, Museu Nacional, Entomological Image Collection / M.V. De Almeida). Lost in the fire of 2018 (Pinto *et al.*, in prep.).

Male measurements (mm, n = 5) Total length (including caudal appendages) 31.0-33.0; abdomen length (excluding caudal appendages) 25.1-28.8; head maximum width 3.5-3.6; Fw length 15.5-17.1; Hw length 14.5-16.6; Fw maximum width 3.6-3.9;

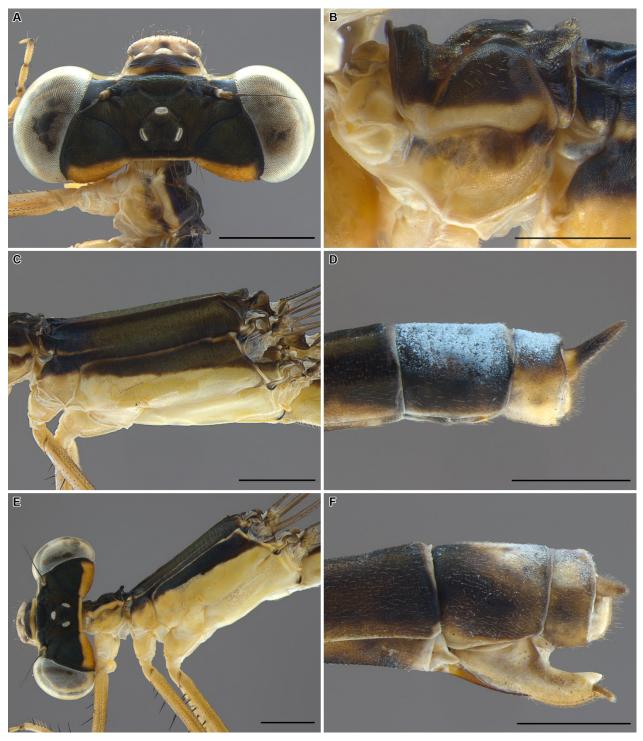


Figure 3. R. beckeri (Brazil. PR: Curitiba, DZUP): (a)-(d) male; (e)-(f) female; (a) head in dorsal view; (b) prothorax in lateral view; (c) synthorax in lateral view; (d) S8-10 and caudal appendages in lateral view; (e) head and thorax in lateral view; (f) S8-10, ovipositor and caudal appendages in lateral view. Scale bars (mm): (a), (c)-(f) = 1; (b) = 0.5.

Hw maximum width 3.5-3.6; pt height length 0.22-0.32 in Fw; 0.22-0.30 in Hw; pt length costal margin 0.12-0.27 in Fw; 0.12-0.25 in Hw; pt length RA margin 0.17-0.32 in Fw; 0.22-0.30 in Hw; length of metathoracic femur 2.6-3.0; metathoracic tibia 2.1-2.9; length of S9+10 in lateral view 1.5-1.7; length of cercus (dorsal branch) in lateral view 0.57-0.65; length of ventrobasal process in lateral view 0.15-0.20.

F e m a l e m e a s u r e m e n t s (m m, n = 5) Total length (including caudal appendages) 33.0-35.0; abdomen length (excluding caudal appendages) 28.2-30.1; head maximum width 3.4-3.5; Fw length 17.7-18.1; Hw length 16.3-18.1; Fw maximum width 4.0-4.4; Hw maximum width 3.9-4.3; pt height length 0.27-0.37 in Fw; 0.27-0.33 in Hw; pt length costal margin 0.15-0.22 in Fw; 0.15-0.27 in Hw; pt length RA margin 0.25-0.4

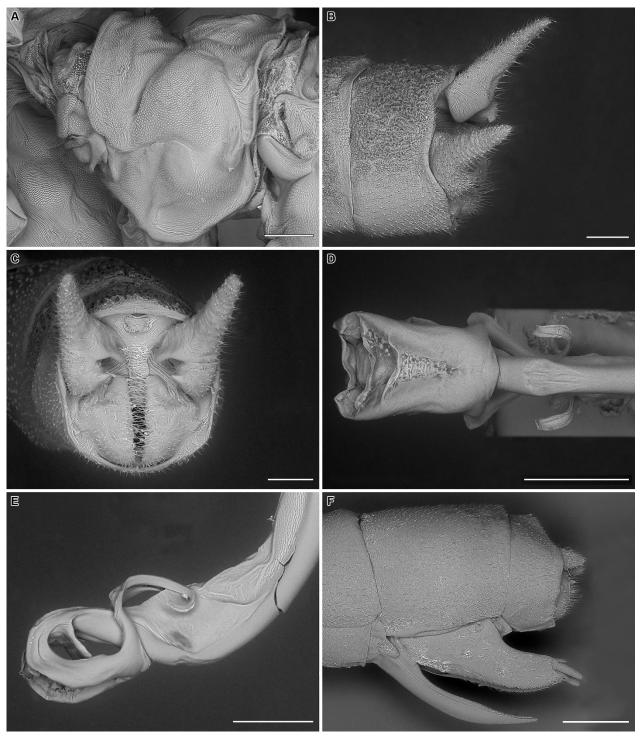


Figure 4. Scanning electron microscope (SEM) images of *R. beckeri* (Brazil. PR: Curitiba, DZUP): (a)-(e) male; (f) female; (a) prothorax in lateral view; (b)-(c) caudal appendages in dorsolateral (b) and posterior (c) views; (d)-(e) genital ligula in ectal (d) and lateral (e) views; (f) S8-10, ovipositor and caudal appendages in lateral view. Scale bars (mm): (a)-(e) = 0.2; (f) = 0.5.

in Fw; 0.20-0.35 in Hw; length of metathoracic femur 2.8-3.1; metathoracic tibia 2.6-3.0; length of S9+10 in lateral view 1.5-1.6; length of cercus in lateral view 0.22-0.30.

Diagnosis

R. beckeri is a medium protoneurine (30-35 mm of length), metallic black dorsally and orange-yellow laterally. The angulated frons; rear of head pale; dorsal margin

of antennifer smooth (uncarinated); Ax space 1 shorter than twice the length of Ax space 2 and shorter than Ax space 3; MP long, extending for more than 2 cells posterior to vein descending from subnodus; CuP and AA fused (indistinct) to wing margin in Fw and Hw; genital ligula (GL) with long inner fold and lateral lobes prolonged to form two long flagella (figures 4d, 4e); cercus slightly forcipate with a very short ventrobasal (VBP) and a robust

mediobasal (MBP) processes (figures 3d, 4b, 4c); posterolateral margin of median lobe of prothorax with a minute, but still discernable tubercle-like projection (figures 3b, 4a); all these characters agree with the generic diagnosis by Garrison *et al.* (2010), and allow for separation of specimens of *Roppaneura* from all the species that belong to other Protoneurinae genera.

Males and females of *R. beckeri* can be diagnosed from all species included in the poorly-supported *Roppaneura* clade (i.e., *Amazoneura* Machado, *Forcepsioneura* Lencioni, *Lamproneura* De Marmels, *Phasmoneura* Williamson, and *Psaironeura* Williamson; see Pessacq, 2008; Pinto and Kompier, 2018) by the following combination of characters: rear of head pale (dark in *Amazoneura* and *Psaironeura*), CuP and AA in Hw indistinct (distinct in *Lamproneura* and *Phasmoneura*), and MP long, extending for more than 2 cells posterior to vein descending from subnodus (short, extending less than two cells in *Amazoneura*, *Forcepsioneura*, *Phasmoneura* and *Psaironeura*).

Specimens of R. beckeri are remarkably similar to those of the sympatric Atlantic Forest genus Forcepsioneura. Males may be confused with Forcepsioneura mainly based on pale rear of head, GL with a long inner fold and lateral flagella, and forcipate cercus with a VBP and MBP, being distinguished from all known species in that genus by: (1) very short VBP as long as ≤ 0.20 of dorsal branch of cercus in lateral view, (2) long MP extended more than two cells distal to vein descending to subnodus (while in Forcepsioneura the VBP as long as ≥ 0.30 of dorsal branch of cercus in lateral view and MP short at most reaches anal margin distally 0.5 from the vein descending from subnodus). Females of R. beckeri may be

distinguished from known females of *Forcepsioneura* by distal tip of stylus reaching the level of the cercus, while in *Forcepsioneura* the distal tip surpasses the tip of the cercus.

Garrison et al. (2010) suggested that the postero-medial margin of postocular lobe almost aligned with occipital margin is a unique character of the genus. However, our study shows this character unsuitable as diagnostic of this genus. Even though the postocular lobe is not protruding as much as in other Protoneurinae genera (e.g., Epipleoneura Williamson), none of the specimens we examined (figure 3a) are similar in appearance to the illustration by Garrison et al. (2010), suggesting that there are geographically varying morphotypes for this character. Rosser Garrison kindly investigated the material in his collection, all from the municipality of Florestal in Minas Gerais (Angelo Machado's "population") and stated "[...] I checked the other five specimens in my collection and can tell you that the head proportions are about the same. My illustration of the head (Fig. 2529) was drawn using a camera lucida so I don't think I made an error in drawing. I can see from your excellent photos that the head does seem to be slightly more constricted medially in your new populations but I think that you both might agree with me that its overall appearance is similar and differs, say, from a majority of other protoneurine genera. Might these differences be the results of morphological variation? [...]" (Rosser Garrison, personal communication). We roughly compared the distance between occipital bar and postocular lobe among R. beckeri populations with species of Forcepsioneura and indeed, the specimens from Paraná appeared to be more similar to some Forcepsioneura species (figure 5).

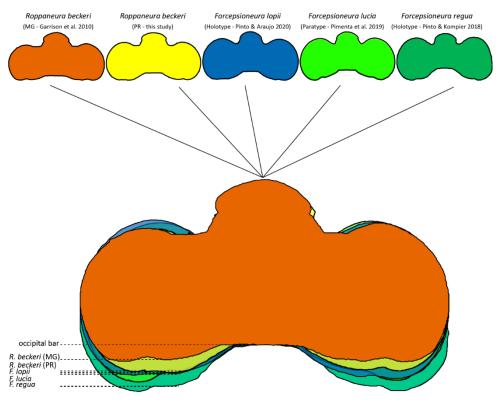


Figure 5. Comparison of outline of the heads of two populations of *R. beckeri* and species of *Forcepsioneura*. Superimposed images aligned by the occipital bar.

Distribution

Endemic to Brazil, occurring between 775 and 1385 meters of altitude in the states of Minas Gerais and Paraná, in the Paraná and Araucaria Forests biogeographical provinces (figure 1).

Remarks

Our study shown that this recently discovered population has dark areas both larger and more intense in comparison with the previously known individuals as depicted below.

Males

Face orange-yellow, head dorsally black (figure 3a). Labrum with a yellow line ventrally, light brown to dark brown at clypeolabral suture prolonged into two lateral ill-defined spots and a mesal pear-shaped dark spot. Anteclypeus with an ill-defined light-brown spot mesally. postclypeus with a dark brown to black transverse band occupying anterior 0.66. Mesal area of antefrons, all postfrons and most of epicranium black with copperygreen metallic reflections; 0.8 of postocular lobe and occipital bar orange-yellow in dorsal view (figure 3a). Synthorax dark areas greater (figures 3b, 3c) dorsally with purplish-blue at 0.2 anterior and coppery-green at 0.8 posterior metallic reflections (figures 3c, 3e). The three yellow stripes on mesothorax are poorly defined, shorter, and narrower (figures 3c, 3e). Abdomen laterally whitish-yellow with noticeable dorsal dark areas and spots with coppery metallic reflections (figure 3d); S1-7 and S10 laterally pale yellow, S8-9 brown to dark brown, S9-10 with whitish-blue pruinosity dorsally. Dorsal of S1-7 brown to dark-brown, with a dark-brown posterior ring at 0.10 of segment length on S4-7, S9 with a pair of laterodorsal black rounded spots on similar to seen in species of Oxyagrion Selys (figure 3d), the anterior pale yellow ring on S3-7 separated dorsally.

Females

The brown coloration at labrum is less clear (figure 3e); dorsal pruinosity on 0.5 of S9 and S10 less dense (figure 3f). First and second valves brown and ventral margin with serration; third valve yellow but 0.9 in the basal region to 0.8 of height with a brown spot, distal region with setae. Stylus brown (figures 3f, 4f).

Discussion

Distributional data on *R. beckeri* is meagre, as well as many aspects of its life history, ecology, and evolution which are still poorly known. Our recording of a population of *R. beckeri* extends its distribution south by 500 km and is the first record to southern Atlantic Forest and state of Paraná, and just the fourth known site of occurrence of this species.

This urban population of *R. beckeri* has an overall darker coloration compared to published data (cf. Santos, 1966; Garrison *et al.*, 2010). Colour variation in insects can be caused by genetic factors (e.g., polygenic, mutation and polymorphism; Sanmartín-Villa and Cordero-Rivera, 2016), phenotypic plasticity (e.g., light, defence

mechanism; Schnitger et al., 2007; Michie et al., 2010; Barnard et al., 2015) or due to ontogenetic colour change (Sanmartín-Villa and Cordero-Rivera, 2016; Joshi and Agache, 2020). Based on Ford's definition of polymorphism, that excludes geographical races and considering the variation in this population does not represent discontinuous forms (morphs) of this species within the same population, polymorphism hypothesis is discarded (see Ford, 1957). Teneral emerged in the laboratory presented the same level of melanisation few hours after emergence as captured fully mature adults, so variation in coloration cannot be explained by ontogenetic changes. Low intensity of ultraviolet radiation (UV) in rearing-laboratory conditions can develop melanism in damselflies of the genus Enallagma Charpentier (see Barnard et al., 2015), but this condition is unlikely because individuals collected in the field (highly exposed to UV) presented the largest dark areas in the body. Causes of melanisation in this population are yet unknown, but we believe it is due to a geographic feature of this population, thus polygeny would be the cause. However, the low levels of UV radiation in comparison to the northern populations could also contribute to explain it and linked to this aspect, another putative explanation would be the thermal melanism hypothesis. Pinkert et al. (2017) identified a positive correlation between higher latitudes and dark coloration, they shown that odonates in the North Hemisphere tend to be darker coloured in regions with cold climate most likely to compensate for the low temperatures because darker coloured individuals absorb more solar irradiance.

R. beckeri figures among the highest priority targets for dragonfly conservation. Moore (1997) ranked R. beckeri as a priority species based on its belonging to a monotypic genus and on its endemicity to a small area in Brazil. However, the association of species of Odonata with small collections of water in or upon plants is an exceptional trait among extant dragonflies and damselflies because less than 1% of the order is known to breed on phytotelmata. The dendrolimnetobiont R. beckeri is just found associated with E. floribundum and has outstandingly unusual biological characteristics. It is considered critically endangered and the fact that the only known living population is in an urban environment is among the reasons for its current status (Pinto et al., 2020). Odonates in urban ecosystems are subjects to stressors like the removal of native vegetation, modification of hydrological systems, "heat islands" and accumulation of pollutants (Villalobos-Jiménez, 2016). Furthermore, R. beckeri is endemic to the Atlantic Forest, a domain under extreme pressure with 88.3% of its original vegetation lost and which continues to be threatened by human activities, with remaining forest cover mostly composed of small fragments (Ribeiro, 2009). The R. beckeri sites are in Minas Gerais and Paraná, states that are leaders in deforestation of the Atlantic Forest between 2016 and 2017 in Brazil (Fundação SOS Mata Atlântica and INPE,

Although studies such as predictive species distribution modeling and on the identification of threats to the ecological and evolutionary processes of *R. beckeri* would be important to the development of a conservation plan,

the current information allows us to strongly suggest that efforts should be focused on protecting this patch of vegetation at Centro Politécnico Campus of UFPR for conservation of this rare damselfly. The species is dependent of individuals of E. floribundum to survive and therefore it also needs the surrounding vegetation and associated marshy soil where these species co-occur. E. floribundum is distributed in Argentina, Paraguay, and Uruguay, while in Brazil it is recorded from the state of Goiás in the Cerrado south to Rio Grande do Sul at the Atlantic Forest and Pampa domains (Flora do Brasil, 2020). Recently E. floribundum was considered Vulnerable (VU) in the state of Paraná (Cardozo, 2017). Further searches within the distribution range of its hostplant, E. floribundum, would hopefully lead to the discover of additional populations of R. beckeri. Some areas near Curitiba have populations of E. floribundum (Cardozo, 2017; Flora do Brasil, 2020) and should be investigated for the occurrence of R. beckeri.

We strongly recommend including R. beckeri as a priority species for dragonfly conservation policies in South America, due to its exclusive biological characteristics, evolutionary relevance, and occurrence in urban to periurban landscapes. Monitoring efforts of this urban population at a university campus are candidate for citizen science-based programs involving students as volunteers. The appeal of damselflies to the general public makes them a flagship species for conservation (Oertli, 2008) and citizen science projects related to conservation issues have more chance to be successful (Frigerio et al., 2017). Indeed, dragonflies as a whole have been largely used to indicate the quality of the freshwater habitats and can act as "guardians of the watershed" (see Clausnitzer et al., 2017). They can serve as both environmental indicators and flagship species; many successful programs for environmental education on water quality linking dragonflies, scientific knowledge, conservation and citizen scientists are known from Asia and Africa (see Clausnitzer et al., 2017).

Even though more studies are needed to fully understand their relationships, some recent studies have shed light on the phylogenetic relationships of the *Roppaneura* clade, and *Roppaneura* was found to be closely related to *Forcepsioneura* (Pimenta, 2019; Pimenta *et al.*, 2019). As further subjects of needed research we suggest the following: (1) what are the generic circumscriptions of these genera (see Pinto and Araujo, 2020), and (2) what evolutionary processes lead from breeding in lotic environments to breeding in phytotelmata.

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