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Patterns of fluorescence in a collection of Paraguayan Didelphidae

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Abstract: Many species of opossum (Didelphimorphia) are known to fluoresce, but the significance of this characteristic is unclear. In the interests of contributing to the faunal inventory of fluorescence in Didelphids, we examined 62 specimens of 10 species of Paraguayan opossums under a UV light and describe the patterns observed. Of particular interest is a clear apparent difference in fluorescence between two cryptic and occasionally sympatric species, *Marmosa rapposa* and *Marmosa paraguayana* which may be a potential isolating mechanism. Furthermore, we suggest the possibility that fluorescence declines with time since collection in *Didelphis albiventris,* and the fluorescence in that species is not related to age or sex. While the significance of fluorescence in Didelphids remains obscure, patterns observed show some degree of species specificity within the geographic boundaries of this study.

Key-words: *Cryptonanus;* Didelphidae; *Didelphis;* Fluorescence; *Lutreolina; Marmosa.*

Resumo: Padrões de fluorescência em uma coleção de Didelphidae paraguaios. Muitas espécies de gambá (Didelphimorphia) são conhecidas por fluorescer, mas o significado dessa característica não é claro. Com o objetivo de contribuir para o inventário faunístico de fluorescência em didelfídeos, examinamos 62 espécimes de 10 espécies de gambás paraguaios sob luz negra e descrevemos os padrões observados. De particular interesse, há uma clara diferença na fluorescência entre duas espécies crípticas e ocasionalmente simpátricas, *Marmosa rapposa* e *Marmosa paraguayana,* que pode ser um potencial mecanismo de isolamento. Além disso, sugerimos a possibilidade de que a fluorescência diminua com o tempo desde a coleta em *Didelphis albiventris,* e que a fluorescência nessa espécie não esteja relacionada à idade ou sexo. Embora o significado da fluorescência em Didelphidae permaneça obscuro, os padrões observados mostram algum grau de especificidade da espécie dentro dos limites geográficos deste estudo.

Palavras-chave: *Cryptonanus;* Didelphidae; *Didelphis;* Flourescência; *Lutreolina; Marmosa.*

INTRODUCTION

The fluorescent reflective properties of certain opossum species (Didelphidae) has long been known (Anderson *et al.,* 1978; Meisner, 1983; Pine, 1978, 1979, 1981; Pine & Handley Jr, 1984; Pine *et al.,* 1985), and recently the coverage of other taxonomic groups showing these properties has increased (Hunt *et al.,* 2009; Gruber & Sparks, 2015; Kohler *et al.,* 2019; Olson *et al.,* 2020, Sobral & Souza-Gudinho, 2022; Weidensaul *et al.,* 2011). The ecological significance of fluorescence remains unclear, but theories have been posited that it acts to influence mate choice (Arnold *et al.,* 2002), to dissuade predators (Olofsson *et al.,* 2010), that it is related to crepuscular activity (Kohler *et al.,* 2019), or is evolutionary by-product that is ecologically functionless (Snyder *et al.,* 2012).

Pine *et al.* (1985) documented fluorescence in 23 species of didelphid but was unable to identify any patterns of age, sex or season within his sample of over 600 specimens (in several mammal collections at the following institutions: National Museum of Natural History, Field Museum of Natural History, Carnegie Museum of Natural History, American Museum of Natural History, Museum of Natural History of the University of Kansas, Los Angeles County Museum of Natural History). Only five of the species examined by Pine that returned a positive result (*Caluromys lanatus, Chironectes minimus, Lutreolina crassicaudata, Metachirus nudicaudatus, Monodelphis domestica*) form part of the Paraguayan didelphid fauna, as well as one other that returned a negative result (*Monodelphis dimidiata* listed as *Monodelphis sorex*). However, the geographic origins of Pine's specimens were not provided and the taxonomy of Didelphidae has undergone considerable changes in the intervening period (and is likely to in the future) (Voss, 2022). With access to a larger sample of Paraguayan didelphid specimens, we examined the UV reflective properties of the different species and report our findings here.

MATERIALS AND METHODS

A handheld LED flashlight was passed over the dorsal and ventral side of the examined didelphid skins within a dark room. First we lit the guard hairs, then lit a second time through the undercoat by separating the fur. The resulting intensity of the fluorescence was then

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ranked using a system from 0-3. Fluorescent intensity was scored by assigning 0 to absence of fluorescence, a ranking of 1 was given if there was a dull purple which was deemed as possible reflection of the UV; 2 was given if there was a mild intensity to the fluorescence (moderate reflectivity) and 3 equaled a high intensity (strong reflectivity). Though these scores are necessarily subjective they were made in direct comparison with other specimens, enabling a reliable degree of scoring. The score represents the overall fluorescent intensity displayed across the whole skin, including both guard hair and undercoat. These scores were agreed upon by both authors for each specimen.

RESULTS

A total of 62 Paraguayan specimens of 10 species of Didelphidae: *Cryptonanus chacoensis* (Tate, 1931), *Chironectes minimus* (Zimmermann, 1780), *Didelphis albiventris* Lund, 1840, *Gracilinanus agilis* (Burmeister, 1854), *Lutreolina crassicaudata* (Desmarest, 1854), *Marmosa paraguayana* (Tate, 1931), *Ma. rapposa* (Thomas, 1899), *Monodelphis domestica* (Wagner, 1842), *Mo. kunsi* Pine, 1975 and *Thylamys macrurus* (Olfers, 1814) were examined with the UV flashlight. Species were identified following the keys in Gardner (2008) and nomenclature was updated were necessary using Voss (2022). Of these, only four species showed evidence of fluorescence: *D. albiventris, L. crassicaudata, Ma. rapposa* and *Ma. paraguayana.* A diagnostic difference was observed in the reflective pattern between the two congeneric species of *Marmosa.* Specimen information and fluorescence observed for the 62 specimens was catalogued (Table 1). Examples and comparisons of the fluorescence observed can be seen in Figure 1a-e, although we were unable to capture an accurate representation of what was observed with the naked eye due to equipment limitations.

DISCUSSION

The difference in apparent fluorescent patterns of *Ma. paraguayana* and *Ma. rapposa* is potentially significant (Table 1). Both species fluoresce, but the dorsal fluorescence is much stronger in *Ma. paraguayana* (scored as 3; n = 3) than in *Ma. rapposa* (which only moderately reflects the UV light, scored as 2; n = 13). The small sample size studied here does not allow firm conclusions to be drawn, but if the difference holds in larger series, then it could potentially be of use as a diagnostic character for identifying these two, rather similar, species in Paraguay (Smith & Owen, 2016). Broadly, *Ma. paraguayana* is associated with the humid Atlantic Forest ecoregion, while *Ma. rapposa* is associated with the subhumid gallery forests of the Cerrado and Humid Chaco ecoregions (Smith & Owen, 2015; 2016). However, the two species are sympatric in at least one locality in Amambay department (Smith & Owen, 2015), and further studies may show greater overlap of the species range.

Why there may be differences in the distribution of the fluorescence in these two congeneric and ecologically-similar species also raises questions about the purpose of the characteristic, with previously posited suggestions such as a relationship to crepuscular activity (Kohler *et al.,* 2019) or dissuasion of predation (Olofsson *et al.,* 2020) being called into question. With both of these species presumably under similar ecological constraints and pressures, the differences exhibited between the two species are intriguing. Another potential hypothesis is that the presence of luminescence in one of two sympatric species is an isolating mechanism that allows the two species to distinguish each other (granting the assumption that the species themselves can see the fluorescent effects) (Blair, 1950). Given that the current extent of geographic overlap between these two species is limited, this may seem like an unlikely explanation, but we do not know enough about the evolutionary histories of these two species to quantify its historic potential as an isolating mechanism. Pine *et al.* (1985) found luminescence to be widespread in the genus *Marmosa* (as currently taxonomically defined) with *Marmosa andersoni* Pine, 1972, *Marmosa mexicana* Merriam, 1897, *Marmosa robinsoni* Bangs, 1898 and *Marmosa simonsi* Thomas, 1899 all showing fluorescence but *Marmosa rubra* Tate, 1931 not showing it. Unfortunately, none of these species occur sympatrically with each other to allow this hypothesis to be tested.

The species sampled by Pine *et al.* (1985) were classified into distinct subgenera by Voss *et al.* (2014), with the fluorescing species *Ma. andersoni* in *Stegomarmosa* Pine, 1972 and *Ma. mexicana, Ma. robinsoni* and *Ma. simonsi* in *Exulomarmosa* Voss *et al.,* 2014, whilst the non-fluorescing *Ma. rubra* was placed in the monotypic subgenus *Eomarmosa* Voss *et al.,* 2014. Whilst this may indicate a taxonomic association with the character, it is of note that the two Paraguayan species are both currently classified in the same subgenus *Micoureus* Lesson, 1842. Unfortunately, no data is available as to the fluorescent characteristics of the other species assigned to that subgenus.

Pine *et al.* (1985) did not detect fluorescence in their "*Didelphis azarae*" (= *D. albiventris*) specimens. Interestingly we detected strong fluorescence of the ventral fur in all our specimens collected after July 2019 irrespective of age and sex. Those collected earlier did not, so there is perhaps a decline in fluorescence with specimen age. However, much older specimens of other species have continued to fluoresce, demonstrating that this phenomenon is likely the result of complex phenomena we do not yet understand (Anich *et al.,* 2020; Kohler *et al.,* 2019; Olson *et al.,* 2021; Sobral & Souza-Gudinho, 2022).

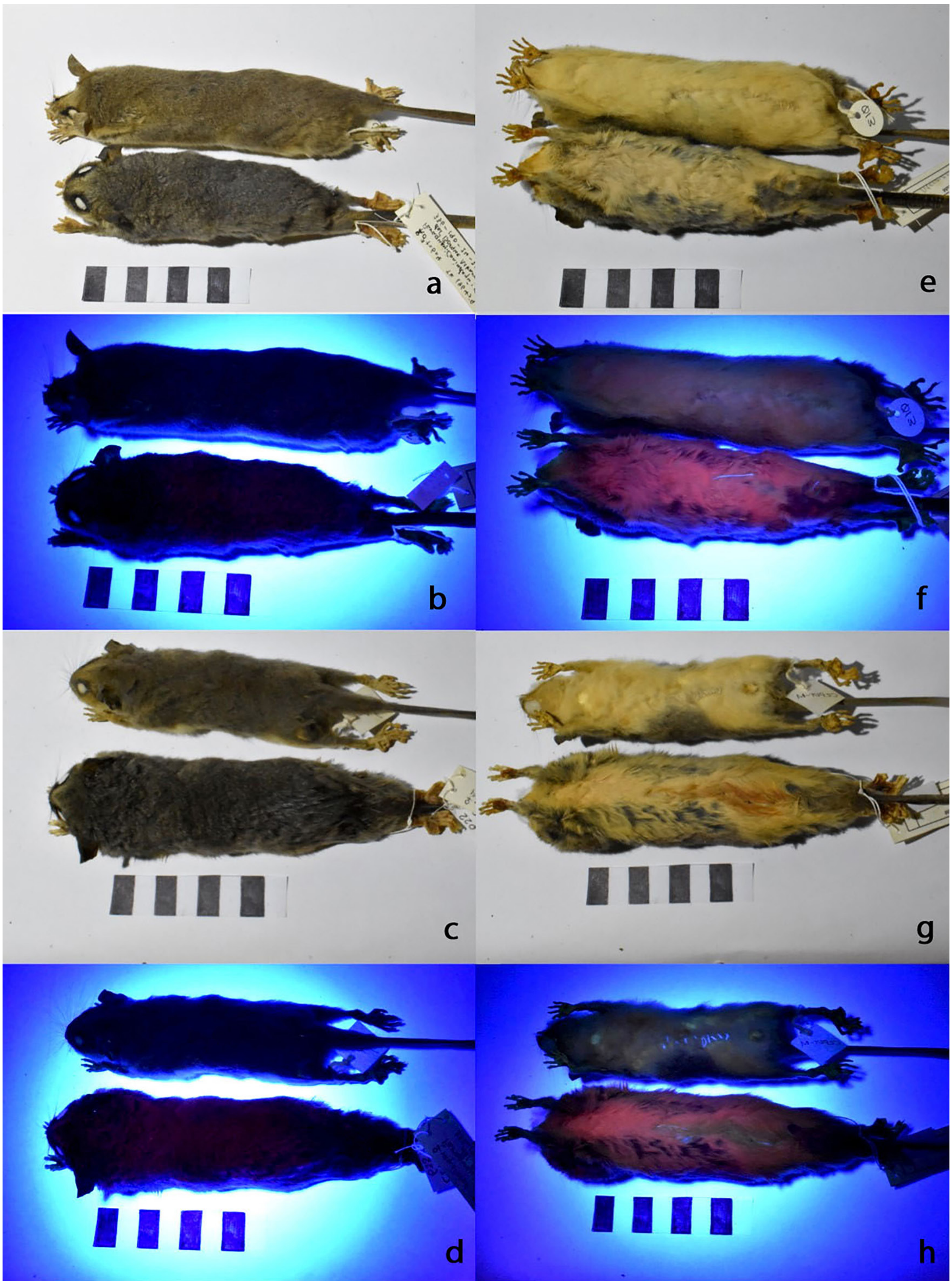

Figure 1. a: *Marmosa rapposa* CZPLT-M-013 (above) and *Marmosa paraguayana* CZPLT-M-650 (below) dorsal view under normal lighting conditions. **b:** *Marmosa rapposa* CZPLT-M-013 (above) and *Marmosa paraguayana* CZPLT-M-650 (below) dorsal view under UV lighting conditions. Neither can be seen responding to the UV light, although CZPLT-M-650 glowed with a strong red when observed with the naked eye. **c:** *Marmosa rapposa* CZPLT-M-206 (above) and *Marmosa paraguayana* CZPLT-M-655 (below) dorsal view under normal lighting conditions. **d:** *Marmosa rapposa* CZPLT-M-206 (above) and *Marmosa paraguayana* CZPLT-M-655 (below) dorsal view under UV lighting conditions. CZPLT-M-206 reflects with a faint purple shine but this was not picked up by the camera. CZPLT-M-655 reflects with a strong red hue but this is only just visible within the photograph. **e:** *Marmosa rapposa* CZPLT-M-013 (above) and *Marmosa paraguayana* CZPLT-M-650 (below) ventral view under normal lighting conditions. **f:** *Marmosa rapposa* CZPLT-M-013 (above) and *Marmosa paraguayana* CZPLT-M-650 (below) ventral view under UV lighting conditions. CZPLT-M-013 only weakly reacts whereas CZPLT-M-650 glows with a strong red hue. **g:** *Marmosa rapposa* CZPLT-M-206 (above) and *Marmosa paraguayana* CZPLT-M-655 (below) ventral view under normal lighting conditions. **h:** *Marmosa rapposa* CZPLT-M-206 (above) and *Marmosa paraguayana* CZPLT-M-655 (below) ventral view under UV lighting conditions. CZPLT-M-206 reflects with a faint purple shine but this was not picked up by the camera. CZPLT-M-655 reflects with a strong red hue.

Table 1: Overview of the analyzed specimen data and the observed fluorescence data. M = male; F = female; U = unsexed.

Catalogue #	Species	Date coll.	Locality	Collector	Fluorescence Observed?	Description of Fluorescence	Fluorescence Scale
CO3125	*Chironectes minimus* U	Unknown	Unknown	J. Contreras	No	No glow	0
CZPLT-M-010	*Didelphis albiventris* M	29.vi.2010	Reserva Natural Laguna Blanca, San Pedro	K. Atkinson	No	No glow, guard hairs not present	0
CZPLT-M-219	*Didelphis albiventris* M	23.iii.2012	Reserva Natural Laguna Blanca, San Pedro	H. Pheasey	No	No glow	0
CZPLT-M-475	*Didelphis albiventris* M	21.v.2016	Reserva Natural Laguna Blanca, San Pedro	H. Davies	No	No glow	0
CZPLT-M-476	*Didelphis albiventris* F	04.iv.2016	null	R. Smith	No	No glow	0
CZPLT-M-516	*Didelphis albiventris* M	01.vii.2018	Ruta 4, E of Pilar, Ñeembucú	R. Smith	No	No glow	0
CZPLT-M-548	*Didelphis albiventris* M	03.vii.2019	14 de Mayo, Pilar, Ñeembucú	B. Pett	Yes	Underfur and ventral fur glow red	2
CZPLT-M-976	*Didelphis albiventris* M	05.xii.2020	Puerto Elisa, Ñeembucú	A. Quinones	Yes	Underfur and ventral fur glow red	2
CZPLT-M-977	*Didelphis albiventris* M	10.xii.2020	Puerto Elisa, Ñeembucú	F. Recalde	Yes	Underfur and ventral fur glow red	2
CZPLT-M-980	*Didelphis albiventris* M	16.xii.2020	Puerto Elisa, Ñeembucú	A. Quinones	Yes	Underfur and ventral fur glow red	2
CZPLT-M-484	*Lutreolina crassicaudata* M	14.x.2017	Ruta 4. 4 km East of Pilar, Ñeembucú	J. Dickens	Yes	Both ventral and dorsal fur glow red. Both upper and under fur.	3
CZPLT-M-657	*Lutreolina crassicaudata* U	31.vii.2019	Avda Gral Santos y Mcal Lopez, Asunción, Central	H. Sanchez	Yes	Both ventral and dorsal fur glow red. Both upper and under fur.	3
CZPLT-M-012	*Marmosa rapposa* M	26.vii.2010	Reserva Natural Laguna Blanca, San Pedro	K. Atkinson	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-013	*Marmosa rapposa* F	30.vii.2010	Reserva Natural Laguna Blanca, San Pedro	K. Atkinson	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-014	*Marmosa rapposa* F	03.viii.2010	Reserva Natural Laguna Blanca, San Pedro	K. Atkinson	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-015	*Marmosa rapposa* F	02.ii.2011	Reserva Natural Laguna Blanca, San Pedro	K. Atkinson	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-206	*Marmosa rapposa* M	09.i.2012	Reserva Natural Laguna Blanca, San Pedro	B. Graham	No	Faint purple shine	1
CZPLT-M-236	*Marmosa rapposa* M	08.ix.2021	Reserva Natural Laguna Blanca, San Pedro	R. Owen	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-256	*Marmosa rapposa* M	24.ix.2012	Reserva Natural Laguna Blanca, San Pedro	R. Owen	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-261	*Marmosa rapposa* F	21.ix.2012	Reserva Natural Laguna Blanca, San Pedro	R. Owen	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2

Catalogue #	Species	Date coll.	Locality	Collector	Fluorescence Observed?	Description of Fluorescence	Fluorescence Scale
CZPLT-M-436	*Marmosa rapposa* M	12.x.2013	Reserva Natural Laguna Blanca, San Pedro	P. Kirby & B. McVeigh	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-437	*Marmosa rapposa* F	10.x.2013	Reserva Natural Laguna Blanca, San Pedro	P. Kirby & B. McVeigh	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-459	*Marmosa rapposa* M	29.viii.2014	Reserva Natural Laguna Blanca, San Pedro	H. O'Donnell	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-478	*Marmosa rapposa* M	07.v.2015	Reserva Natural Laguna Blanca, San Pedro	J. Hogg	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-490	*Marmosa rapposa* M	29.viii.2016	Reserva Natural Laguna Blanca, San Pedro	K. Atkinson	Yes	Upper fur only weakly glows, likely reflection. Underfur and ventral glows red	2
CZPLT-M-650	*Marmosa paraguayana* F	22.ii.2017	Reserva de Biosfera del Bosque Mbaracayú, Canindeyú	R. Owen	Yes	Both ventral and dorsal fur glow red. Both upper and under fur.	3
CZPLT-M-653	*Marmosa paraguayana* F	13.ii.2017	Reserva de Biosfera del Bosque Mbaracayú, Canindeyú	R. Owen	Yes	Both ventral and dorsal fur glow red. Both upper and under fur.	3
CZPLT-M-655	*Marmosa paraguayana* U	23.vi.2015	Reserva de Biosfera del Bosque Mbaracayú, Canindeyú	R. Owen	Yes	Both ventral and dorsal fur glow red. Both upper and under fur.	3
CZPLT-M-204	*Gracilinanus agilis* M	09.i.2012	Reserva Natural Laguna Blanca, San Pedro	B. Graham	No	Faint purple glow	1
CZPLT-M-205	*Gracilinanus agilis* F	09.i.2012	Reserva Natural Laguna Blanca, San Pedro	B. Graham	No	No glow	0
CZPLT-M-207	*Gracilinanus agilis* M	09.i.2012	Reserva Natural Laguna Blanca, San Pedro	B. Graham	No	Faint purple shine	1
CZPLT-M-208	*Gracilinanus agilis* M	09.i.2012	Reserva Natural Laguna Blanca, San Pedro	B. Graham	No	Faint purple shine	1
CZPLT-M-209	*Gracilinanus agilis* M	11.i.2012	Reserva Natural Laguna Blanca, San Pedro	B. Graham	No	Faint purple shine	1
CZPLT-M-210	*Gracilinanus agilis* F	25.i.2012	Reserva Natural Laguna Blanca, San Pedro	B. Graham	No	Faint purple shine	1
CZPLT-M-253	*Gracilinanus agilis* F	21.ix.2012	Reserva Natural Laguna Blanca, San Pedro	R. Owen	No	Faint purple shine	1
CZPLT-M-424	*Gracilinanus agilis* F	23.i.2013	Reserva Natural Laguna Blanca, San Pedro	R. Owen	No	Faint purple shine	1
CZPLT-M-443	*Gracilinanus agilis* U	27.viii.2014	Reserva Natural Laguna Blanca, San Pedro	H. O'Donnell	No	Faint purple shine	1

Catalogue #	Species	Date coll.	Locality	Collector	Fluorescence Observed?	Description of Fluorescence	Fluorescence Scale
CZPLT-M-446	*Gracilinanus agilis* M	05.viii.2014	Reserva Natural Laguna Blanca, San Pedro	H. O'Donnell	No	Faint purple shine	1
CZPLT-M-448	*Gracilinanus agilis* M	20.viii.2014	Reserva Natural Laguna Blanca, San Pedro	E. Briggs	No	Faint purple shine	1
CZPLT-M-450	*Gracilinanus agilis* M	19.ix.2014	Reserva Natural Laguna Blanca, San Pedro	K. Atkinson	No	Faint purple shine	1
CZPLT-M-457	*Gracilinanus agilis* F	19.xii.2014	Reserva Natural Laguna Blanca, San Pedro	H. O'Donnell	No	Faint purple shine	1
CZPLT-M-022	*Cryptonanus chacoensis* F	09.ix.2011	Reserva Natural Laguna Blanca, San Pedro	H. Pheasey	No	Faint purple shine	1
CZPLT-M-217	*Cryptonanus chacoensis* F	14.iii.2012	Reserva Natural Laguna Blanca, San Pedro	H. Pheasey	No	No glow	0
CZPLT-M-965	*Cryptonanus chacoensis* M	03.xii.2020	Puerto Elisa, Ñeembucú	J. Ayala	No	Faint purple shine	1
CZPLT-M-973	*Cryptonanus chacoensis* M	08.xii.2020	Puerto Elisa, Ñeembucú	A. Quiñones	No	No glow	0
CZPLT-M-974	*Cryptonanus chacoensis* F	04.xii.2020	Puerto Elisa, Ñeembucú	J. Ayala	No	No glow	0
CZPLT-M-979	*Cryptonanus chacoensis* M	16.xii.2020	Puerto Elisa, Ñeembucú	F. Recalde	No	No glow	0
CZPLT-M-986	*Cryptonanus chacoensis* M	24.ii.2021	Santa Ana, Ñeembucú	S. Krabbendam	No	No glow	0
CZPLT-M-988	*Cryptonanus chacoensis* M	25.ii.2021	Santa Ana, Ñeembucú	K. Armstrong	No	Faint purple shine	1
CZPLT-M-989	*Cryptonanus chacoensis* M	26.ii.2021	Santa Ana, Ñeembucú	S. Krabbendam	No	No glow	0
CZPLT-M-990	*Cryptonanus chacoensis* M	02.iii.2021	Santa Ana, Ñeembucú	K. Armstrong	No	No glow	0
CZPLT-M-1019	*Cryptonanus chacoensis* M	12.iii.2021	Santa Ana, Ñeembucú	S. Krabbendam	No	No glow	0
CZPLT-M-1036	*Cryptonanus chacoensis* M	16.iii.2021	Santa Ana, Ñeembucú	K. Armstrong	No	No glow	0
CZPLT-M-1044	*Cryptonanus chacoensis* M	19.iii.2021	Santa Ana, Ñeembucú	S. Krabbendam	No	No glow	0
CZPLT-M-1063	*Cryptonanus chacoensis* F	07.iv.2021	Santa Ana, Ñeembucú	K. Armstrong	No	Faint purple shine	1
IBIS 1231	*Cryptonanus chacoensis* U	08.vi.1994	Itapúa	F. Colman	No	No glow	0
CZPLT-M-011	*Monodelphis domestica* M	17.vii.2010	Reserva Natural Laguna Blanca, San Pedro	K. Atkinson	No	Faint purple shine	1
CZPLT-M-030	*Monodelphis domestica* M	30.xii.2011	Reserva Natural Laguna Blanca, San Pedro	H. Pheasey	No	Faint purple shine	1
CZPLT-M-005	*Monodelphis kunsi* M	24.xi.2011	Reserva Natural Laguna Blanca, San Pedro	H. Pheasey	No	Faint purple shine	1
CZPLT-M-215	*Monodelphis kunsi* M	23.ii.2012	Reserva Natural Laguna Blanca, San Pedro	H. Pheasey	No	Faint purple shine	1
CZPLT-M-234	*Monodelphis kunsi* F	17.v.2012	Reserva Natural Laguna Blanca, San Pedro	R. Burger	No	Faint purple shine	1
	Thylamys macrurus U	N/A	Cordillera de los Altos		No	No glow	0

Pine *et al.* (1985) included *Mo. domestica* amongst the species that did fluoresce (22 of 28 specimens), but neither of the two specimens of this species in our collection exhibited the character. All of these specimens showed a dull purple "reflection" which we do not consider fluorescence under the definition employed here. A similar effect was seen in our specimens of the congeneric *Mo. kunsi* which was not sampled by Pine.

The significance of fluorescence in Didelphids continues to be obscure, and there are no observable patterns of occurrence that can be attributed to the family at this stage. Fluorescence seems to show specific distribution in some species, whilst in others patterns are variable and cannot be associated with age or sex. That there may be some decline in fluorescence with specimen age in some species (*e.g., Didelphis albiventris*) is worthy of further study, as is the potential for fluorescence to be used as a diagnostic tool in some cryptic species.

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