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Distribution of a residual population of the Dytiscid *Graphoderus bilineatus* (de Geer, 1774) in the Grande Cariçaie nature reserves, Switzerland

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Abstract

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Introduction

Out of the 13 Dytiscid species of the genus Graphoderus (Holmgren et al. 2016), four species (G. bilineatus; Figure 1), G. cinereus (Linnaeus, 1758), G. zonatus (Hoppe, 1795) and G. austriacus (Sturm, 1834)) can be found in Switzerland (Carron 2005). All four species are threatened locally in many regions of their global distribution range (e.g. Hendrich and Müller 2017); G. bilineatus is of particular concern. Even though the species shows a wide distribution and is encountered in most European countries, it is rare, and its distribution is very fragmented. G. bilineatus is declining, particularly in the western range of its distribution (Holmen 1993), and is considered extinct in several countries i.e., Belgium (Scheers 2015) and the United Kingdom (Foster 1996). The IUCN Red List (Foster 1996) indexed it as "vulnerable" and the species is now protected in most European countries, following the Appendix II of the Bern Convention (Council of Europe 1979). In Switzerland, G. bilineatus was first

Currently, the distribution of diving beetles in Switzerland is poorly known making it difficult to determine conservation priorities for species with small and/or declining populations. In order to establish conservation priorities, in 2018, we surveyed diving beetles of the genus *Graphoderus* in the Grande Cariçaie reserves with special consideration for the Red Listed *G. bilineatus*. While *G. bilineatus* and *G. cinereus* showed high habitat niche overlap, the distribution of *G. bilineatus* was limited to mainly one of the eight reserves. When comparing our results to available historical data, the habitat of *G. bilineatus* has likely diminished during the last 40 years. Our study provides the first comprehensive documentation of the distribution of *Graphoderus* species in the Grande Cariçaie. We further highlight the importance for improving the knowledge of *G. bilineatus* distribution in Switzerland to develop policy for conservation of this globally threatened species.

> listed as vulnerable in the former Red List of endangered animal species established in 1994 (Duelli et al. 1994). Nevertheless, as the family of Dytiscids has since not been studied for an updated Red list, *G. bilineatus* ' status is now unclear. As a consequence, the species does not stand on the national priority species list (OFEV 2011).

> *G. bilineatus* is known from several locations in Switzerland, but of the available data, only two locations refer to observations made after 2000 (Info fauna - CSCF). Several observations (of at least five individuals) were made near Wetzikon, in the canton of Zürich in 2008 and 2009 (Carron 2009, Info fauna -CSCF) while all other observations are restricted to the Grande Cariçaie nature reserves. It is to be noted that the country's museum collections have not yet been completely inventoried regarding Dytiscids, resulting in a possibility of additional observations. In 1973 and 1974, Brancucci (1979; 1980) undertook a diving beetle inventory in the Motte reserve, one of the eight reserves comprising the Grande Cariçaie. His study revealed

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Figure 1. *Graphoderus bilineatus*. Copyright: Yerpo [CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0)], from Wikimedia Commons.

densely localised populations of *G. bilineatus* and *G. cinereus*. *G. bilineatus* is currently the only aquatic insect listed as priority species in the reserves, where it was rediscovered in 2001. Since then, the species has been observed irregularly in the reserves. In order to estimate the current distribution of the population of *G. bilineatus* we sampled the southern shore of Lake Neuchâtel. We thereafter compared the beetle's distribution with historical data to evaluate population trajectory. Finally, we compared *G. bilineatus*' distribution and habitat niche with those of *G. cinereus* to understand how these two species might cohabitate.

Materials and methods

Study site

The study took place in the Grande Cariçaie marshland nature reserves, Switzerland (Figure 2). The Grande Cariçaie is composed of eight reserves (2,300 hectares), spread across 40 km along the south eastern shore of Lake Neuchâtel. It is the largest lakeside marshland of the country and hosts a considerable amount of national and European priority species. The marshland arose following the first Jura water correction in 1870s, which aimed to better regulate the Aar river and reduce the risk of flooding in the surrounding areas. Following these corrections, the large marshland lowlands of the Seeland region (deep depression area at the foot of the Jura mountain chain, in which lie the lakes of Neuchâtel, Biel and Morat) dried out. The water level of Lake Neuchâtel decreased by about three meters, revealing a large portion of the lake floor on which today's marshland is found. The resulting wetland thus played the role of reservoir for many species that depend on these particular habitats. Given the level of the lake has since been artificially regulated, the Grande Cariçaie marshland is not subject to strong natural dynamics of floods and droughts, resulting in a natural succession to scrub and woodland of the area. Since the end of the 1970s, the area is under constant management for its preservation.

Sampling

In 2018, nine habitat types were sampled (Table 1) and stations (each ~50 m²) were selected following a stratified purposeful design. 101 stations were designated along the southern shore of Lake Neuchâtel and sampled between May 7th and July 3rd, corresponding to part of the main estimated reproduction period (highest activity) of the priority species G. bilineatus (Brancucci 1980). In order to facilitate sampling, the stations were clustered into units of four to six geographically close stations, which were visited on the same day. We sampled the station clusters in a randomised order. The low number of sampling stations in the reserve of Grèves de la Corbière et de Chevroux (Figure 1) is due to the limited number of favourable habitats for diving beetles present in the area, which is mainly composed of forest and reed beds (Phragmites australis; (Cav) Trin. ex Steud., 1841).

For each station, the following measures were taken: mean depth of the water in a radius of two meters around each trap, percentage of helophyte cover, percentage of hydrophyte cover, presence/pseudo-absence of fish as well as presence/pseudo-absence of fish fry. The latter two were assessed opportunistically through sightings during the installation and retrieval of the traps, accidental captures and existing knowledge of Antoine Gander. All stations were located in open areas without shading.

We sampled the beetles by the means of two complementary methods (Hilsenhoff and Tracy 1985), using baited bottle-traps and macrofauna nets. The bottle-traps were built from 1.5 L PET bottles of which the top was cut off and replaced in the bottle upside down, creating a funnel (entry surface $\sim 100 \text{ cm}^2$). Inside each bottle, we placed fresh pork liver as bait for the beetles (Kalnins 2006, Koese and Cuppen 2006). Each trap was attached to a stick, which was pegged in the soil to maintain it near the surface of the water. This ensured that the opening was kept under water and the bottle contained enough air for the beetles to breathe. This sampling method was proved to be efficient for capturing large to medium sized species from the family Dytiscidae, or Hydrophilidae beetles (Hilsenhoff 1987, Kalnins 2006, Koese and Cuppen 2006). Six bottles were placed in each sampling site, distributed in different microhabitats (e.g. open water, different vegetation types) on site



Figure 2. Location of the sampling stations in the Grande Cariçaie nature reserves, on the south eastern shore of Lake Neuchâtel. We sampled seven reserves in 2018 (delimited in brown polygons): 1) Grèves de Cheseaux, 2) Baie d'Yvonand, 3) Cheyres, 4) Grèves de la Corbière et de Chevroux, 5) Grèves d'Ostende et de Chevroux, 6) Grèves de la Motte and 7) Cudrefin. The orange points represent the sampled stations (N = 101 stations). Background picture obtained from the Swiss Federal Office of Topography swisstopo.

Table 1. Graphoderus sp. captures per habitat type in 2018. N° st. = number of stations of the given habitat type in which the species
was captured. N° ind. = number of individuals captured in a given habitat and percentage of the total amount of individuals of that
species captured in the corresponding habitat. Habitat types are ordered from the most permanently flooded habitat to the driest,
with reed bed soil stripping referring to reed beds where ~30cm of the organic layer was removed as to recreated flooded areas and
meadow referring to non-permanently flooded wet meadows.

	G. bilineatus		G. cinereus		G. zonatus	
Habitat type	N° st.	N° ind.	N° st.	N° ind.	N° st.	N° ind.
Pond	4	6 (38%)	8	23 (25%)	1	1 (25%)
Reed bed soil stripping	0	0	7	18 (19%)	0	0
Reed bed	0	0	1	6 (6%)	0	0
Rut	1	1 (6%)	2	5 (5%)	0	0
Carex elata (Koch 1926) meadow	0	0	8	21 (22%)	0	0
Cladium mariscus ((L.) Pohl, 1809) and C. elata meadow	1	2 (12.5%)	3	6 (6%)	0	0
C.mariscus meadow	0	0	1	1 (1%)	0	0
C. mariscus and Carex panicea (L., 1753) meadow	1	2 (12.5%)	1	1 (1%)	0	0
Schoenus nigricans (L., 1753) meadow	3	5 (31%)	6	14 (15%)	2	3 (75%)
TOTAL	10	16	37	94	3	4

and left overnight (~20 hours). When recovering the traps, we sorted the adult specimens in order to release all those of the genus *Graphoderus*, after having determined the species and sex of each individual. Collection

and identification of beetles in the field was done by Antoine Gander, Aline Knoblauch and Khalil Outemzabet. Individuals for which identification was not certain were collected and identified in the lab with the help of a stereo microscope. Identification of these specimens was later confirmed by Albertine Roulet. Specimens will be stored at the Cantonal Museum of Zoology in Lausanne. Two individuals of *G. bilineatus* and *G. zonatus* as well as three individuals of *G. cinereus* are kept in the collection of the Association de la Grande Cariçaie as reference specimens.

Given the monitoring was part of a larger inventory project, each station was equally sampled with a standard macrofauna net to capture smaller species as well as species that would not react to bait found in the bottle-traps (Hilsenhoff 1987, Koese and Cuppen 2006). We emptied the material caught in the net in a white tray in which we did the sorting. We performed a minimum of five short net swipes (duration \approx 5 seconds, area \approx 1m² per net swipe), while aiming at different microhabitats. As long as new diving beetle morph types were recognized, we continued sampling (for further details see Carron 1999; Carron et al. 2007).

Collection of historic data

Additionally, we collected historic observations from 1936 (one site south east of the Grèves de Cheseaux reserve), 1948 (one site at the same location as 1936 and one site between the Baie d'Yvonand and Cheyres reserves), 1949 (one site, same location as 1936 and 1948), 1974 (Brancucci 1979; 1980), 2001 (one site in the Motte reserve), and 2014 from the AGC data bank.

In 2014, 41 stations lying in the Motte and the Grèves d'Ostende et de Chevroux reserves were sampled using bottle traps only, between May 27^{th} and June 20^{th} . *G. bilineatus* was captured in four stations lying in the Motte reserve (seven beetles; Figure 3) and in three stations lying in the Grèves d'Ostende et de Chevroux reserve (nine beetles). In the same year, 49 *G. cinereus* were captured in 14 stations lying in the Motte reserve (38 beetles) and in seven stations lying in the Grèves d'Ostende et de Chevroux reserve (17 beetles). Three *G. zonatus* were captured in one station of the Motte reserve.



Figure 3. Stations in which *Graphoderus bilineatus* was captured in the Motte reserve in 2014 (green) and in 2018 (yellow). The dotted lines represent the reserve boundaries and the grey points the sampled stations (2018) in which no *Graphoderus bilineatus* were captured. Background picture obtained from the Swiss Federal Office of Topography swisstopo.

Data analysis

All analyses were conducted in R version 3.3.1 (R Core Team 2018). We used the unpaired two-sided Wilcoxon test to compare the medians of the three habitat measurements (depth, helophyte and hydrophyte cover) between the stations where G. bilineatus and G. cinereus were captured and where the species were not captured. We performed a principal component analysis (PCA) to compare the niches of G. bilineatus and G. cinereus regarding depth, helophyte and hydrophyte cover of the habitats. For the analysis of niche breadth and niche overlap, we used the R package spaa (Zhang 2016). Both analyses were computed based on the proportion of Graphoderus beetles captured in the nine sampled habitat types (Table 1). Niche breadth was calculated using Levin's measure of niche breadth (Levins 1968) and standardized to express the values on a scale going from 0 (no resources used in common) to 1 (complete overlap) (Hurlbert 1978).

Results

Out of the 101 stations sampled, specimens of the genus *Graphoderus* were captured in 41 of them (41% of the stations; Figure 4). Among these stations, *G. bilineatus* and *G. cinereus* were captured simultaneously in only six stations, while *G. zonatus* was once captured with both other *Graphoderus* species and twice with *G. cinereus* only. *Graphoderus* sp. were present in the following reserves: Cudrefin, Motte, Grève d'Ostende et de Chevroux, Grève de la Corbière et de Chevroux, Cheyres, and Grèves de Cheseaux. We did not capture any individuals of the genus *Graphoderus* using macrofauna nets.

Graphoderus bilineatus

Fifteen G. bilineatus (5 males and 10 females) were captured between May 17th and June 19th in 10 out of the 101 sampled stations (10%). A maximum of three individuals were captured together in one station. The population seems to be concentrated in the Motte reserve (11 specimens; presence in 19% of the 42 sampled stations; Figures 2, 4a) and in the Grèves d'Ostende et de Chevroux reserves (4 specimens, present in 7% of the 29 sampled stations; Figure 4b). The species was captured in various habitat types listed in Table 1. Median depth of the stations in which G. bilineatus was captured was 25 cm, median helophyte cover -58%, and median hydrophyte cover -3%. The medians of these three measures did not differ significantly from those of stations in which the species was not detected (two-sided Wilcoxon test, W_{depth} = 412.5, p-value_{depth} = 0.529, W_{helophyte cover} = 347.5, p-val-ue_{helophyte cover} = 0.223, W_{hydrophyte cover} = 422.5, p-value_{hydrophyte} = 0.709). Presence of fish were recorded for only one station in which G. bilineatus was detected and no fry were recorded.

Graphoderus cinereus

Ninety-four G. cinereus were captured in 37 stations (37% of all sampled stations), of which 26 males, 66 females and 2 of unidentified sex (28%, 70%, 2% respectively). They were trapped between May 7th and July 2nd. Most of the individuals were captured in the Grèves d'Ostende et de Chevroux reserve (61%; Figure 4b), some in the Motte reserve (28%; Figure 4a), and a few in the Grèves de la Corbière et de Chevroux (4%), Cudrefin (3%), Cheyres (2%) and Grèves de Cheseaux (2%) reserves. The various habitats in which G. cinereus were captured are listed in Table 1. Median depth of the stations in which G. cinereus was captured was 21 cm, median helophyte cover - 75%, median hydrophyte cover -1%, and median for both fish and fry presence -0. The medians of these measures did not differ significantly from those of stations in which the species was not detected (two-sided Wilcoxon test, W_{depth} =1107, p-value_{depth} = 0.37, W_{helophyte cover} = 1232.5, p-value_{helo} = 0.735, W_{hydrophyte cover} = 1181.5, p-value_{hydrophyte} cov-er = 0.989, W_{fish} = 1299, p-value_{fish} = 0.322), except for the presence of fry. G. cinereus was significantly more found in stations in which no fry had been detected ($W_{fry} = 1009$, p-value_{frv} = 0.033). Measures from stations in which G. cinereus was found did not significantly differ from those of stations in which G. bilineatus was captured ($W_{depth} = 196.5$, $\begin{array}{l} \text{p-value}_{\text{depth}} = 0.774, \text{W}_{\text{helophyte cover}} = 236, \text{p-value}_{\text{helophyte cover}} = 0.188, \text{W}_{\text{hydrophyte cover}} = 199.5, \text{p-value}_{\text{hydrophyte cover}} = 0.709). \end{array}$

Graphoderus zonatus

Four *G. zonatus* were captured in three stations (3% of all sampled stations), of which three males and one female, between May 17^{th} and May 30^{th} . One specimen was captured in the Grèves de Cheseaux reserve and three were captured in the Motte reserve. The habitat types in which *G. zonatus* was captured are listed in Table 1. The measurements of the three stations in which the species was captured were, respectively, as following: helophyte cover 60%; 50%; 90%, hydrophyte cover 0%; 3%; 0% and depth 15 cm; 40 cm; 24 cm. Given the low number of individuals captured, we did not include the species in further analysis.

Niche analyses

The PCA results did not reveal a clear clustering between *G. bilineatus* and *G. cinereus*, which largely overlap (Table 2, Figure 5). The first principal component (PC1), explain-

Table 2. Factor loadings of the three principal components (PC) based on habitat measures for *G. bilineatus* and *G. cinereus*. The highest loadings for each component are in bold.

Measurement	PC1	PC2	PC3
Helophyte cover	0.556	-0.790	-0.256
Hydrophyte cover	-0.598	-0.168	0.783
Depth	-0.576	-0.589	-0.566
Standard deviation	1.328	0.816	0.754
Proportion of variance	0.588	0.222	0.190
Cumulative variance	0.588	0.810	1



Figure 4. Stations in which *Graphoderus bilineatus* (yellow), *G. cinereus* (red) and *G. zonatus* (blue) were captured: A) in the Motte reserve and B) in the Ostende reserve in 2018. The dotted lines represent the reserve boundaries and the grey points the sampled stations in which no *Graphoderus* were captured. Background picture obtained from the Swiss Federal Office of Topography swisstopo.



Figure 5. Principal component (PC) for habitat depth, helophyte cover and hydrophyte cover for *G. bilineatus* (green) and *G. cinereus* (blue). The first principal component (PC1) explains 59% of the variance. The first two components (PC1 and PC2) explain 81% of the total variance. While factor loads of habitat depth, helophyte cover and hydrophyte cover for PC1 are similar, helophyte cover and depth strongly negatively influence PC2. Circles represent 95% probability ellipses.

ing 59% of total variance, was negatively correlated with depth and hydrophyte cover, while being positively correlated with helophyte cover. PC2, explaining 22% of total variance, was negatively correlated with helophyte cover as well as with depth and hydrophyte cover. The third PC, explaining 19% of the variance, was positively correlated with hydrophyte cover. The standardized niche breadths (NB) indicate that *G. cinereus* has a wider habitat niche than *G. bilineatus* (NB_{*G. cinereus*} = 0.511, NB_{*G. bilineatus*} = 0.292). Niche overlap between both species is high (O = 0.68).

Discussion

Within the Grande Cariçaie, the main pool of *G. bilineatus* seems to be limited to the Motte reserve. Historical data suggests there might have been small populations in the south western part of the lake's shore, even though misidentification cannot be excluded. While we captured 11 *G. bilineatus*, 26 *G. cinereus* and 4 *G. zonatus* in the Motte reserve, Brancucci (1979, 1980) in his inventory of the same reserve mentions high densities of *G. bilineatus* in the sampled area, with 174 individuals captured from mid-March to mid-August 1974 (Brancucci 1978). The same goes for *G. cinereus* with 162 captured individuals, while no *G. zonatus* were captured. Brancucci (1980) classifies both *G. bilineatus* and *G. cinereus* as frequent and abundant in ponds. Given the protocols between Brancucci's study and ours are different, it is not possible to

estimate population trajectory during the last 40 years. Indeed, Brancucci's study consisted of continuous trapping throughout the whole sampling period, in five ponds (area $200-500 \text{ m}^2$). In each of the studied ponds he set ten baited cage traps (five of surface entry = 225 cm^2 and five of surface entry = 100 cm^2), which were emptied every third day.

Since Brancucci's study (1979, 1980), the natural maturation of the habitat led to siltation of several water bodies or humid areas – among which the ponds he sampled. The reduction or disappearance of these areas could impact the already very localised populations of Graphoderus. Vegetation succession and biocenotic evolution are indeed mentioned as one of the principal threats or pressures to G. bilineatus in the report on the species drawn from the European Environment Agency 2007-2012 (EIONET 2012). In response to the loss of waterbodies, soil stripping - the action of removing the organic layer over approximatively 30 centimetres – might be considered to maintain favourable habitats for the beetle by restoring flooded areas. However, in some areas of the Motte reserve, this solution raises management conflicts for the conservation of another priority species as diggings could also favour the expansion of the invasive Pelophylax (Fitzinger, 1843) frogs, which supposedly compete with the protected Green tree frog Hyla arborea (Linnaeus, 1758). The Motte reserve is the only reserve from the Grande Cariçaie to be almost free of the Pelophylax frogs (Leuenberger 2013). Therefore, it should be avoided to support their expansion by creation of new water bodies in those areas. Invasive alien species are another threat stated in EIONET (2012) which should be monitored in the Grande Cariçaie in the future. The decimation of 98% of the diving beetle species by the Louisiana crawfish Procambarus clarkii (Girard, 1852) in the Perges marsh in France, including that of G. bilineatus (Bameul 2013), perfectly illustrates the importance of regular control and surveillance of exotic species for rare species conservation. Finally, dispersal of fish, potential predators of Graphoderus beetles, from Lake Neuchâtel to ponds that are or might become linked to the lake will be monitored in the near future.

G. bilineatus and G. cinereus supposedly share the same diet (Deding 1988, Cuppen et al. 2006) and phenology (Brancucci 1980), but otherwise little is known about their ecology. Both species were described as sedentary through a mark-recapture study by Brancucci (1980), in which they also shared a similar spatial distribution, and do not show significant differences in flight ability (Iversen et al. 2017). Our results reveal a segregation of G. bilineatus and G. cinereus in the adjacent Motte and Grèves d'Ostende et de Chevroux reserves. Both species supposedly share similar needs in terms of habitat types (high niche overlap) and measures (depth, helophyte and hydrophyte cover, Figure 5) and show comparable dispersal abilities (Iversen et al. 2017). Two hypotheses might explain this difference in distribution: (1) a level of competition for an ecological niche between both species, highlighted by the rarefication of vital habitats, G.

cinereus being more successful than *G. bilineatus*, or possibly (2) a difference in sensitivity to habitat reduction. *G. bilineatus* might have shared *G. cinereus*' habitats in both reserves at some point but being more sensitive to habitat loss its population shrank at a faster pace than that of *G. cinereus*. In our study, *G. bilineatus* indeed showed a narrower habitat niche than *G. cinereus*. However, our knowledge on *Graphoderus* ecology needs to be developed in order to make further assumptions.

Iversen et al. (2013) investigated the wide geographical distribution of G. bilineatus in Sweden, Estonia and Poland, linked to its low dispersal ability. The authors suggest that specificity of habitat characteristics is not vital for the species, which shows a wider ecological niche than previously thought - also illustrated by the large variety of habitats in which the species was captured in the Grande Cariçaie. The presence of the species rather depends on landscape connectivity, distance to a possible source habitat as well as stability of the site (Iversen et al. 2013). Given the low dispersal ability of G. bilineatus (Brancucci 1980, Iversen et al. 2017), the species is likely to depend on the availability of dispersal corridors to widen its distribution. It would then, as suggested by Iversen et al. (2013), be wise to concentrate conservation efforts not only on creating and maintaining favourable habitats, but also towards the problematics of landscape structure, through linear dispersal corridors, which showed greater success than stepping stones.

Switzerland nowadays probably lies on the south-eastern limit of G. bilineatus' distribution range (EIONET 2012) and shows very fragmented wetland habitats due to intensification of agriculture and high demographic density. Since 1950, about 90% of wetland areas have disappeared (Klaus 2007). This results in all wetland habitats being classified as vulnerable (Delarze et al. 2016). In Switzerland, given the low habitat connectivity on a large scale, priority lies in preserving and reinforcing local populations of G. bilineatus. In the Grande Cariçaie, connectivity between reserves is interrupted by the presence of villages or towns on the shore of the lake. Furthermore, the Motte reserve differentiates from the others by a higher amount of permanently flooded zones of little depth (e.g. flooded meadows). Interestingly, recent records (after 2000) of G. bilineatus originate from the same locations as some of another rare priority invertebrate species, Nehalennia speciosa (Charpentier, 1840; Odonata): the Motte reserve and Wetzikon (Gander 2010; Info fauna - CSCF). The similar localised distribution of both species in the Grande Cariçaie can eventually support the idea of the Motte reserve differing from the rest of the Grande Cariçaie regarding habitat suitability for the two species. This hypothesis is additionally supported by the localised presence of the rare G. zonatus (Carron 2005) in the same reserve. Hence, further investigations would need to be done in order to appreciate the suitability of other reserves' habitats for G. bilineatus and understand how dispersal can be encouraged.

A national inventory combined with inventories of museum collections could be used to describe the largely under-studied Dytiscidae fauna in Switzerland (Carron 2005). A thorough knowledge of G. bilineatus' distribution is essential to establish an appropriate conservation management plan for the beetle. Given the ecological niche of the species is relatively wide and the central pool of its distribution lies at higher latitudes (i.e. Sweden, Latvia; EIONET 2012), prospection should not disregard mountainous areas. As of today, the closest known location where the species is established lies in the French Jura, at 850 m of altitude, 40 km away from our study site (Lambert 2017). The G. bilineatus population of the Grande Cariçaie is possibly one of the last residual pool from the ancestral Seeland population and the above mentioned factors need to be considered for the preservation of the population.

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