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The status of *Protea caffra* subsp. *caffra* and *Harmonia axyridis* in the habitat of the Critically Endangered Pennington's Protea butterfly *Capys penningtoni*

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Abstract: Aerial photography provides historic record of features at the time the photograph was captured, and can assist with exploring changes in the habitat of threatened species. The host-plant of the Critically Endangered, endemic Pennington's Protea butterfly *Capys penningtoni*, the Common Sugarbush *Protea caffra* Meisn. subsp. *caffra*, is a resprouter after fire which eventually dies if burnt too frequently. Six sites (Marwaqa Nature Reserve, Impendle Nature Reserve, Clairmont Mountain Nature Reserve, Lotheni Nature Reserve, Lot 93 1821 near Mkhomazi River [Nxamalala Traditional Council] and Mt Le Sueur) where the butterfly species had been previously recorded were selected to estimate the number of adult *P. caffra* subsp. *caffra* trees present using aerial photographs (2009). Protea trees in the vicinity of the *C. penningtoni* butterfly collection sites were point-digitised off aerial photographs. Polygons were digitised around coverages of protea trees using a distance of 50 m between trees to determine the outer margin of each coverage. The imagery data were ground-truthed by verifying the location and identities of *P. caffra* subsp. *caffra* trees at each site, using a sample of the total number of digitised trees. The ground-truthing enabled the separation of *P. caffra* subsp. *caffra* trees from Silver Protea *Protea roupelliae* trees, and the separation of *P. caffra* subsp. *caffra* trees with overlapping canopies. Contemporary presence of *P. caffra* subsp. *caffra* trees in relation to presence in 2009 was determined. Fire frequency data for each site was obtained via landsatlook viewer (USGS). We found that the protea savanna at most of the sampled sites was burnt about once every year between July and September. This project indicated the potential utility of aerial photographs and aerial imagery for the purposes of assessing the status of *P. caffra* subsp. *caffra* and *H. axyridis* in the habitat of the Critically Endangered and endemic *C. penningtoni* and for directing the placement of sampling plots in the field.

Key words: Critically Endangered species, alien invasive species, butterfly host plant, aerial imagery digitisation, ground-truthing, Protea trees, fire frequency.

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INTRODUCTION

The conservation status of the Critically Endangered Pennington's Protea butterfly *Capys penningtoni* has rapidly deteriorated over time (Henning & Henning, 1989; Henning *et al*., 2009; Mecenero *et al*., 2013; Armstrong, 2016). This butterfly species only occurs in the midlands and Drakensberg foothills of central KwaZulu-Natal, and it spends its whole life on or near its host plant, the Common Sugarbush *Protea caffra* (Henning & Henning, 1989). Two of the apparent major threats to the survival of *C. penningtoni* is the impact of current fire regimes on the survival of its host plant and the potential predation of its early life history stages by the alien invasive Harlequin Ladybird Beetle *Harmonia axyridis*.

Protea caffra survives fire by means of its thick bark and ability to re-sprout again after being burnt (Adie *et al.*, 2011). However, the indigenous encroacher bracken fern *Pteridium aquilinum* (and probably the alien invasive American bramble *Rubus cuneifolius*) growing under and near *P. caffra* may cause fires to burn intensely under the proteas, thereby burning the protea canopies and causing the eventual death of the proteas. Even adult *P. caffra* will eventually die if burnt often enough with intensive fires (Adie *et al.*, 2011). Many parts of the grasslands of central KwaZulu-Natal are burnt annually and this poses a separate problem in that protea seedlings need to grow for several years until they escape the fire trap. Smith and Granger (2016) showed that juvenile silver proteas *Protea roupelliae* take 17 years on average to escape the fire trap.

Protea caffra seedlings have not been observed by us over the past few years at sites where *C. penningtoni* has been recorded. A possibility is that the fire frequency and intensity of fire in the protea savanna where *C. penningtoni* occurs is such that no *P. caffra* seedlings survive long enough to escape the fire trap. Another explanation of this observation is that P. caffra only recruit from the seed under specific environmental conditions. (Hylton, Adie pers. comm.). Adie et al. (2011) considered

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that a regular fire interval of two to three years without the influence of patches of *P. aquilinum* would allow for *P. caffra* persistence in the landscape.

Remote sensing tools have been widely used for assessment and monitoring of biodiversity. The precision of these tools have made it possible to look beyond the mapping of habitat extent (Nagendra, 2001; Boyd & Foody, 2011). Advanced methods of using remote sensing tools include direct mapping of biodiversity, mapping trees and identification of different tree species (Turner *et al*., 2003; Nagendra *et al*., 2012). By using very high spatial resolution imagery one can accurately identify tree canopies (Agarwal *et al*., 2013). The higher rainfall grasslands of the central KwaZulu-Natal midlands and Drakensberg foothills are largely treeless except for scattered tree clumps around boulders and tree proteas established in suitable areas. Therefore remote sensing could be used to determine the distribution and density of tree proteas in this region. A research project was developed to determine how the extinction of *C. penningtoni* could be prevented. An aim of the present study was to determine whether the appropriate siting of sampling plots to assess the status of *P. caffra* at sites where *C. penningtoni* had been recorded was possible before fieldwork commenced, which could also assist with future research. A second aim was to determine the fire history of the sites to be sampled to determine whether *P. caffra* is recruiting or merely persisting or declining in the habitat of *C. penningtoni*.

Harmonia axyridis is originally from the central and eastern parts of Asia, and has the ability to tolerate a wide range of climate (Majerus *et al*., 2006; Brown *et al*., 2011). After the introduction of *H. axyridis* as a bio-control agent in North America and Europe, it has now adversely spread into new environments (Majerus *et al*., 2006; Brown *et al*., 2011; Roy *et al*., 2016). The diet of the omnivorous *H. axyridis* includes the eggs and larvae of Lepidoptera species (Koch *et al*., 2003; Roy *et al*., 2016). *Harmonia axyridis* is classified as a category 1b invader in the South African National Environmental Management: Biodiversity Act No. 10 of 2004, which is an invasive species controlled by an invasive species management programme. Alien invasive species are of global significance as a threat to biodiversity, which calls for effective control measures and management plans. In South Africa, the invasive *H. axyridis* was first recorded in the Western Cape during the early 2000s and has spread quickly to all nine South African provinces (Stals & Prinsloo, 2007; Stals, 2010). *Harmonia axyridis* has multiple impacts in ecosystems, for example outcompeting and predating on native ladybird species (Stals & Prinsloo, 2007). The third aim of this study was to calculate the density of *H. axyridis* in the habitat of *C. penningtoni* in readiness for a potential mass trapping campaign. Mass trapping methods could be developed to possibly save *C. penningtoni* from *H. axyridis*-induced extinction, should the eggs, larvae and/or pupae of *C. penningtoni* be susceptible to predation by the beetle.

This study therefore had the following objectives, to:

1. map the extent of *P. caffra* savanna at some sites where *C. penningtoni* has been recorded, using aerial photographs and aerial imagery;
2. determine the number of adult *P. caffra* trees occurring at each of the sites from the aerial photographs and subsequent ground-truthing of the maps;
3. assess the factors that cause differences in the numbers of proteas counted on the aerial photographs and satellite imagery in comparison with the numbers counted on the ground;
4. determine the fire frequency at each of these sites using remote sensing, and;
5. estimate the density of *H. axyridis* per unit of *P. caffra* savanna vegetation using a direct observation method.

MATERIAL AND METHODS

Delimitation of protea savanna areas where *Capys penningtoni* is known to have occurred

Capys penningtoni records were extracted from the Ezemvelo KZN Wildlife Biodiversity Database. The geographical co-ordinates on the WGS 84 datum of the records were saved as a comma delimited Excel worksheet and then imported into the geographical information system QGIS and saved as a shapefile.

The positions of *P. caffra* trees were digitised off GeoTIFF aerial photograph imagery (of 2 m resolution, collected in 2009; Fig. 1) before going on the ground to assess the accuracy of the identification of *P. caffra* and to identify which *P. caffra* trees had been killed or had died in the interim. We assumed that only protea trees would be visible in the aerial photographs, and not protea shrubs or seedlings. *Protea caffra* and the much less abundant Silver Protea *P. roupelliae* can co-occur and cannot be distinguished using aerial photography, so were combined during digitisation. Protea trees in the vicinity of the *C. penningtoni* butterfly collection spots were then point-digitised by selecting one protea tree at a time, at a manually set map scale of 1: 977.

After point-digitising all the visible protea trees, the distances between the outermost protea trees were measured (m), and various distances subjectively trialled to create a rule for digitising polygons that encompass discrete groups of proteas. The rule finally applied was to create polygons around protea trees that form discrete patches where the outer protea trees were considered those that were within a distance of ≤ 50 m of the next protea tree. A polygon was then digitised using the outer protea trees as the points to represent the outer margin of each patch (Fig. 1). The protea patches were digitised at a map scale of 1: 1 000.

Calculation of *Protea caffra* tree numbers

The areas (m^2) of digitised patches (coverages, Cov) were determined and recorded. The number of protea trees per digitised coverage was counted. The Ezemvelo KZN Wildlife Protected Area boundary [EKZNW_PA_bnd_2017_wdd.shp] and Stewardship [Stewardshp_2017_wdd.shp] shapefiles were used to determine whether the digitised protea coverages were legally protected or not. Maps of the digitised protea coverages were created and exported in PDF and JPEG formats.

Figure 1 – Delimited patches of *Protea caffra* (red dots) from 2009 aerial imagery near the spot where a *Capys penningtoni* specimen had been recorded previously.

Ground-truthing of *Protea caffra* tree densities and status

Six of the 13 sites where *C. penningtoni* was known at the time were selected as the sites for the research project (Table 1), and therefore for ground-truthing the results of the desktop study. Prior to the field visits, map images for the specific location to be visited were colour printed at A3 size.

Three 20 m x 20 m (0.04 ha) plots were to be set up per site. The placement of study plots could be selected based on the protea extents (patch areas) and *P. caffra* tree counts within the coverages (Fig. 2). Generally, ground-truthing was done within the confines of the study plots owing to time constraints. The A3 paper maps were aligned with the aspect of the view. The positions and number of digitised proteas were compared with the positions and number of proteas on the ground. Proteas missing on the ground that could be seen on the aerial photographs and the position and number of different species counted as proteas were all noted on the maps (Fig. 3). Thereafter, the number of proteas on the ground that matched those identified on the images were ticked on the maps and each tree was assessed for fire damage. If there were missing or else extra protea trees on the ground in comparison to those identified as such on the maps, on-site evaluation was done to determine the reasons for the disparity.

Fire frequency at the sampling sites

The Land Satellite Viewer (https://landsatlook.usgs.gov/viewer.html) was used to determine the fire frequency at the six sampling sites. The satellite viewer was accessed from the 3rd to 13th October 2017. The fire frequency was determined by looking at the satellite images per year over a period of 15 years, from 2003 to 2017. The collected data included which month the sites were burnt, how many burns per year and the total number of burns over 15 years.

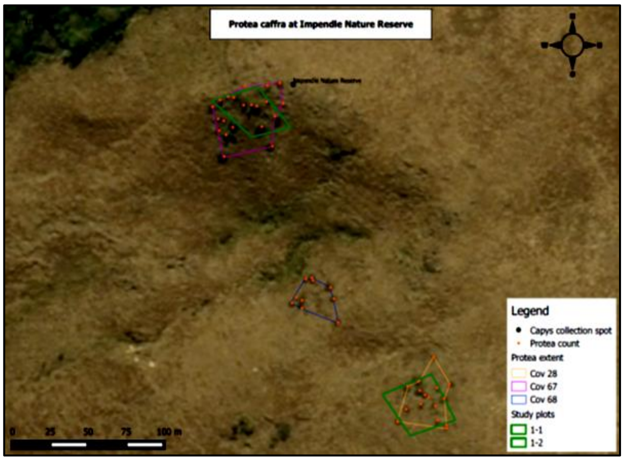

Figure 2 – *Protea caffra* patches and tree positions used to direct the potential placement of sampling plots at Impendle Nature Reserve to estimate *P. caffra* recruitment and resprouting rates (the third plot was at a fourth patch not visible in this figure).

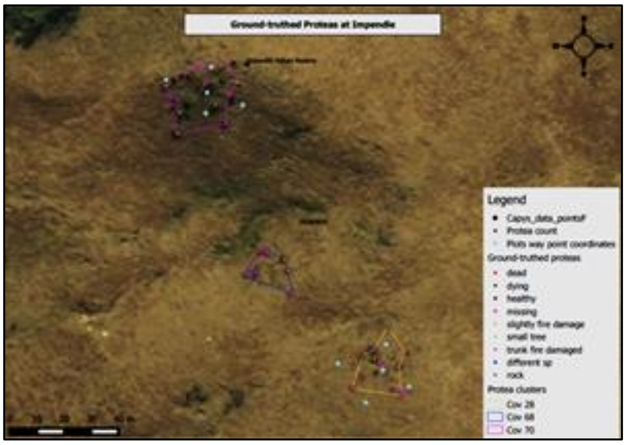

Figure 3 – Ground-truthed *Protea caffra* trees at Impendle Nature Reserve. The imagery map shows the status of the trees per digitised patch (protea cluster coverage; Cov).

Harmonia axyridis densities

The same six sites were chosen for visual counting of *H. axyridis*. Sampling was carried out in three plots of 5 x 5 m (Fig. 4) along a belt transect at each site during the early summer of 2018. Potential double counting of individuals was minimised by setting up the plots as far apart as feasible on the ground. Sampling was carried out on the 5th to the 7th of September 2018 in the Impendle Nature Reserve, the 10th to the 12th of September at Lot 93 1821, the 9th to the 11th of October at the Impendle Nature Reserve, the 15th to the 19th of October at the Marwaqa and Clairmont Mountain Nature Reserves, the 29th to the 31st of October at Mt Le Sueur, and the 12th to the 15th of November at Lotheni Nature Reserve.

RESULTS

Delimitation of protea savanna areas where *Capys penningtoni* is known to have occurred

The size of *P. caffra* patches and the number and density

Table 1 – Estimated number of *Protea caffra* trees at six sites. Protection status of the site, area of each protea patch, and whether one or more plots were to be set up within the protea patch are indicated.

Coverage (Patch)	Site	Status	Tree count	Patch Area (ha)	Plot?
Cov 3	Marwaqa Nature Reserve	Protected	98	0.879	Yes
Cov 4			47	0.883	No
Cov 5			70	0.694	Yes
Cov 14			221	2.435	Yes
Cov 21			17	0.547	No
Cov 85	Mt Le Sueur	Unprotected	167	3.423	Yes
Cov 86			250	3.442	Yes
Cov 28	Impendle Nature Reserve	Protected	14	0.04	Yes
Cov 67			17	0.06	Yes
Cov 68			8	0.010	No
Cov 70			14	0.12	Yes
Cov 71			6	0.010	No
Cov 72	Lot 93 1821	Unprotected	116	1.07	Yes
Cov 83			33	0.084	Yes
Cov 84			51	0.186	Yes
Cov 64	Lotheni Nature Reserve	Protected	471	3.877	Yes
Cov 22	Clairmont Mountain Nature Reserve	Recently protected	686	10.853	Yes

Figure 4 – *Harmonia axyridis* sampling plot (5 x 5 m) at Marwaqa Nature Reserve.

of *P. caffra* trees in each differed between the sites, regardless of whether a site had protected area status or not (Table 1). These data also varied in terms of the patches at the same site. For example, for Impendle Nature Reserve, coverage 28 and 67 had more trees (14 and 17) as compared to coverage 68 with a minimum number of 8 *P. caffra* trees (Table 1).

The potential siting of the three 0.4 ha plots per site could be determined from the size of the *P. caffra* patches and the *P. caffra* tree count of each, subject to the number of available patches at a site (Table 1).

Ground-truthing of *Protea caffra* tree densities and status

Ground-truthing results are shown in Table 2. Some plots had the same number of *P. caffra* trees digitised from the aerial imagery as were found on the ground, those being Mt Le Sueur Plot A (27), Marwaqa Nature Reserve Plot B (13) and Lotheni Nature Reserve Plot B (12). Other plots had differences between the number digitised and the number observed on the ground. There were some *P. caffra* trees seen on the ground that could not be picked up from the aerial imagery. For example, Marwaqa Nature Reserve Plot C had nine *P. caffra* trees digitised off the imagery but 18 *P. caffra* trees were counted on the ground, and Lot 93 1821 Plot B had four digitised *P. caffra* trees but there were 15 on the ground (Table 2). Some *P. caffra* trees that had overlapping canopies could not be resolved into constituent trees, such as in Lotheni Nature Reserve Plot C. Some other tree species (such as *P. roupelliae*) and big rocks were incorrectly digitised as *P. caffra*. For example, Clairmont Mountain Nature Reserve Plot C had a total of 25 *P. caffra* trees digitised on the imagery, but only ten were seen on the ground because eight were dead and seven of the 25 trees digitised were *P. roupelliae*.

An assessment of fire damage on *P. caffra* (Fig. 5) revealed that a number of *P. caffra* trees were dead as a result. Some *P. caffra* trees at all visited sites were moderately to severely fire damaged. A mean of 2 dead *P. caffra* trees per plot were recorded at Impendle Nature Reserve, Marwaqa Nature Reserve and Clairmont Mountain Nature Reserve.

Fire frequency at the sampling sites

The fire frequency data are presented in Table 3. The Mt le Sueur site received the highest number of burns (13) while the Impendle Nature Reserve sites had the fewest fires (9) over the 15 years. Season of burn was consistent in August and September at the Mt Le Sueur and Lot 93 1821 sites but was variable at the other sites. Dead proteas were found mostly in sites that were burnt in June and July (mid-winter), namely Marwaqa and Clairmont Mountain

Nature Reserves, and at the Impendle Nature Reserve site where the burn season was variable.

Table 2 – The number (#) of *Protea caffra* identified on the aerial imagery versus the number of *P. caffra* on the ground. NR = Nature Reserve.

Site and Plot #	# *P. caffra* digitised off imagery	# *P. caffra* on the ground
Impendle NR Plot A	9	10
Impendle NR Plot B	10	9
Impendle NR Plot C	8	8
Lot 93 1821 Plot A	8	9
Lot 93 1821 Plot B	4	15
Lot 93 1821 Plot C	8	10
Lotheni NR Plot A	6	13
Lotheni NR Plot B	12	12
Lotheni NR Plot C	6	16
Mt le Sueur Plot A	27	27
Mt le Sueur Plot B	13	13
Mt le Sueur Plot C	14	12
Marwaqa NR Plot A	7	10
Marwaqa NR Plot B	13	13
Marwaqa NR Plot C	9	18
Clairmont Mountain NR Plot A	6	9
Clairmont Mountain NR Plot B	15	18
Clairmont Mountain NR Plot C	25	10

Figure 5 – Evidence of fire damage of *Protea caffra* tree branches and trunks at A) Clairmont Mountain Nature Reserve; B) Marwaqa Nature Reserve; C) Impendle Nature Reserve; and D) Lotheni Nature Reserve.

Harmonia axyridis density estimation

No *H. axyridis* were recorded from all the plots. Casual observation outside the plots also did not result in any *H. axyridis* records at most of the sites.

DISCUSSION

This study investigated the use of aerial photography to map and determine the distribution and number of *P. caffra* trees at six sites where the Critically Endangered *C. penningtoni* had been recorded. The extent of patches of *P. caffra* at the sites could be recorded, and generally the positions of the majority of *P. caffra* could be plotted, although some *P. caffra* trees could not be picked up from the imagery. The results indicated that recent aerial imagery could be used to decide where to place sampling plots on the ground before going into the field. Also, an increase or decrease in numbers of *P. caffra* trees and sizes of patches over time could conceivably be tracked using a time series of aerial images at certain sites, but not all. This could allow tracking of *P. caffra* population sizes and changes in *P. caffra* distribution over time at the sites, and if combined with monitoring of *C. penningtoni* population sizes and fire history, could provide insight into a potential major cause of decline in the numbers of *C. penningtoni*, that of the impact of fire on the host plant and probably on the mortality of immature stages of the butterfly.

There were various reasons for the discrepancies between the number of *P. caffra* trees that were digitised from the aerial imagery and the number determined from ground-truthing. These included the difficulty of distinguishing (or indeed invisibility of) some of the *P. caffra* trees on some of the imagery, including separating individuals that had overlapping canopies, and separating *P. caffra* trees from some other species of trees, particularly *P. roupelliae*, and large rocks. Agarwal *et al*. (2013) noted that the mapping of trees with different canopy sizes, overlapping canopies and trees of different ages may be extremely difficult. Finally, the considerable difference between the year in which the imagery was taken (2009) and the year in which the ground-truthing was done (2017) meant that some *P. caffra* trees had died in the interim.

The fire history of a site had a visible impact on the *P. caffra* population present. Although *P. caffra* trees escape fire mortality by means of the thick bark and hidden buds, they can be killed by fire. Fire damage was evident on most of the *P. caffra* trees observed during the ground-truthing exercise. Some *P. caffra* trees were dying and some were dead because of the fire damage. Annual burning, which occurred on average at nearly all the sites (Table 3), is detrimental to the vegetation type where *C. penningtoni* occurs; burning should not be more frequent than biennial on average (Joubert *et al*., 2014; Smith & Granger 2016), and perhaps should be more infrequent than that (SANBI, 2014). February (1994) reported a decline of protea savanna in the Drakensberg region and linked this to the increase of veld fires resulting from farming practises. Poultney (2014) reported on the increase of *P. caffra* populations on land where the land use was conservation management. He argued that controlled fire burn systems with appropriate long fire intervals could allow recruitment of *P. caffra* and for juveniles to escape the fire trap. High intensity fires cause mortality in re-sprouting species while cool burns promote re-sprouting (Adie *et al*., 2011). Although burns are crucial in rangeland habitats, Bond & Midgley (2003) and Adie *et al*. (2011) stated that understanding the effects of fire intensity, frequency and timing remain a critical challenge to the fire ecology community. Fuel loads increase over time in the habitat of *C. penningtoni*, and with the presence of alien invasive plants or indigenous encroacher plants growing under the canopies of the host plant trees, intense fires may result (Adie *et al*., 2011).

Table 3 – Fire frequency at the six sampling plot sites for over 15 years. NR = Nature Reserve.

Site	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	Total burn
Lotheni NR	Aug	Jun & Sept	Aug	Jul	Jun	Aug	Jul	Jul	Aug	Sept	Aug	Aug	-	Jul	-	**14**
Mt Le Sueur	Aug	Sept	Aug	Aug	Sept	Aug	-	Aug	Aug	Jun & Sept	Aug	Sept	Sept	Aug	Sept	**15**
Marwaqa NR	Aug	-	Aug	-	Jun	Sept	-	Jul	Jul	Jul	Aug	-	Sept	Aug	Jul	**11**
Impendle NR	-	Sept	Aug	Aug	Aug	Sept	-	Aug	-	Aug	Aug	Sept	-	-	-	**9**
Clairmont Mountain NR	Aug	-	Aug	-	Jun	Sept	Jul	Jul	Jul	Jun	Jun	-	-	Jul	Jun	**11**
Lot 93 1821	Aug	Aug	Aug	Sept	Jun	Sept	-	Aug	Aug	Jun	Aug	Sept	-	-	Sept	**12**

Uncontrolled fires are also more likely in the mountainous habitat of *C. penningtoni* where fickle winds are present. Intense fires in June and July may kill *P. caffra* trees and the larvae and pupae of *C. penningtoni* in the flower buds prior to the adults eclosing in August and September, something that needs to be investigated further. Deliberately started intrusive fires which cannot be controlled as a result of the environmental conditions present and the lack of resources are an increasingly common problem in the protected areas where *C. penningtoni* was previously recorded. Such fires are a major current threat to the survival of the species.

Harmonia axyridis was not found on the *P. caffra* in the plots at the six sites investigated, with no *H. axyridis* beetles observed outside the plots at most of the sites. At Mt Le Sueur, three *H. axyridis* were observed on *P. caffra* outside the plots. This finding indicates that there has been a major crash in the numbers of this invasive alien species in the habitat of *C. penningtoni* before the early summer of 2018. The reasons for this crash are unknown as yet. *Harmonia axyridis* may change host plant use because of food availability, as a number of them were observed on scrambling rose bushes in the farm garden at Mt le Sueur. Brown *et al.* (2011) confirmed that the alien invasive *H. axyridis* beetle is well adapted to garden habitats.

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