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A snapshot of extinction in action: The decline and imminent demise of the endemic *Eligmocarpus* Capuron (Caesalpinioideae, Leguminosae) serves as an example of the fragility of Madagascan ecosystems

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ABSTRACT

The southeastern Madagascan endemic and monotypic genus *Eligmocarpus* is highly threatened due to a combination of factors. Firstly, general human-induced habitat destruction and fragmentation has degraded the environment in which it occurs, leading to an increased threat of extinction for itself and other co-occurring species. Secondly, and more specifically to *Eligmocarpus*, the desirable properties of its timber, which is an excellent construction material, has led to over-collection beyond levels of sustainability. Thirdly, and with the highest relevance for this project, it is a combination of mode of dispersal, germination and seedling establishment. For all these reasons, its range has contracted and the only remaining population (21 trees) is located in Petriky, a future mining site. In this study we investigate the phylogeography and population dynamics of *Eligmocarpus* based on molecular tools (not only conducted on extant individuals but also using herbaria preserved DNA from individuals from neighbouring populations which are no longer alive, to give a glimpse of the past). Prior to human colonisation, the species was successful in using the river network to invade several biomes (most likely from the humid to subarid, where it is now constrained). Hence, due to its location, Petriky is a mosaic of the genetic variability from populations higher up in the river network, therefore, despite the low number of remaining individuals, all hope of restoration is not lost. Within this project we hope that a more complete understanding of the evolution of the flora will allow conservation, not only of current patterns of variation, but also the processes that gave rise to these patterns.

1. Introduction

Madagascar is located in the south-western Indian Ocean and has been recognized as a biodiversity hotspot by Myers et al. (2000) (Fig. 1A). The island is home to 11 220 species of vascular plants, >80% of which are endemic (Callmander et al., 2011). Although Madagascar is facing unprecedented rates of deforestation due to human activities (<10% of primary vegetation remains; Moat and Smith, 2007), botanical expeditions conducted in remote areas still result in the discovery and description of new species (e.g. the Galoka and Kalabenono massifs in North-West Madagascar; Callmander et al., 2009). This is especially true for Leguminosae since the genus studied here, *Eligmocarpus* Capuron, was discovered only 62 years ago (but formally published by Capuron, 1968) and is now facing extinction.

Leguminosae is the third most species-rich family of the Madagascan flora (after the Orchidaceae, ca. 850 spp.; Rubiaceae, ca. 650 spp.; Callmander et al., 2011) with more than 600 species, 70% of which are endemic (Du Puy et al., 2002). In addition, 23 of the 113 genera occurring in Madagascar are restricted to the island (Buerki et al., 2013; Du Puy et al., 2002). Although the family occurs mainly in the dry and subarid biomes, representatives are also found in the humid biome (Du Puy et al., 2002; see Buerki et al., 2013 for more information on the biome distributions and their establishment through time). In this study we focus on the threatened endemic and monotypic genus *Eligmocarpus* (Caesalpinioideae), which is restricted to the south-east of Madagascar (Fig. 1), and has been demonstrated by Bruneau et al. (2008) to be sister to another Madagascan endemic genus, *Baudouinia* Baill. *Eligmocarpus cynometroides* Capuron occurs in the transition zone between the humid and subarid biomes, more specifically between the Andohahela national park (NP) and Petriky (representing a distribution area of <50 km^2; Fig. 1A). Population sizes are always very small, with the biggest population found in Petriky (a future mining site) with <30 trees (Randriatafika et al., 2007). As an example of the decline of this species, 27 trees were recorded in Petriky in 2001 and only 23 trees remained in 2004 (Randriatafika et al., 2007). Moreover, when we visited this site in 2012 two more trees had been felled and several trees are now

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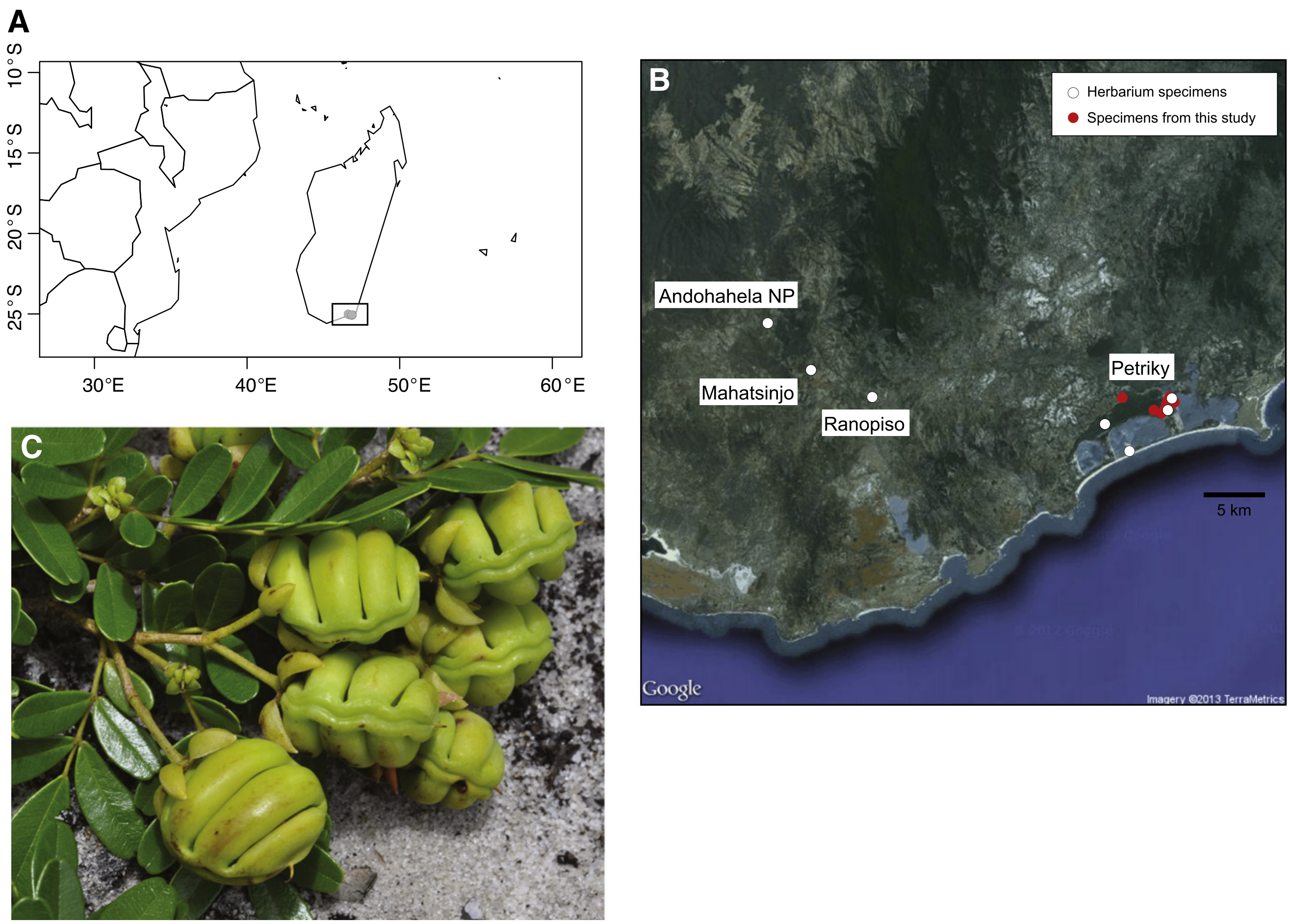

Fig. 1. A. Distribution of *Eligmocarpus cynometroides* in Madagascar. B. Close-up of the distribution of individuals in the Southeast of Madagascar. C. Picture of the fruits of *E. cynometroides* (Buerki et al. 272). [Photograph taken by F. Forest].

surrounded by degraded forest and in the vicinity of villages (Fig. 1). Collection trips, undertaken by the Service Forestier in the 1950s and 1960s, in the forests west of Ranopiso, identified further individuals, but subsequent trips, undertaken by Ratovoson in 1999 were unable to find any trees. This is further evidence that the only viable population today is most likely to be found in Petriky (Ratovoson, pers. comm. 2013). In addition to being threatened by anthropic factors (burning for charcoal, fire, agriculture and construction; Moat and Smith, 2007), this species is widely used by local communities as a timber since its wood is similar to rosewood (*Dalbergia* spp.; Randriatafika et al., 2007). In addition, with the exception of the population found in the Andohahela NP (that might be reduced to only one tree; Ratovoson, pers. comm. 2013), the other individuals occur outside of the national park network and are therefore highly threatened. Outside of Petriky, no individuals of *E. cynometroides* have been collected since 1999 (Table 1). An ecological study conducted on the individuals in Petriky showed that this species has a very low rate of seed production (ca. 1 seed/kg of fruit) and, moreover, its seed germination was also shown to be limited (<5%) (Randriatafika et al., 2007).

In this study we use molecular techniques [DNA sequencing of the Internal Transcribed Spacer (ITS) region and Amplified Fragment Length Polymorphism (AFLP)] to investigate the phylogeography of the species and to assess the genetic variability of the Petriky population (presumably the only remaining population of this species). Results are discussed in light of morphological characters, past climate change and landscape uses. We also extrapolate from our findings to propose a rigorous conservation programme for this species and hopefully to ensure its long-term survival. Finally, we would like to use *E. cynometroides* as a flagship species in order to seek additional funding to protect the unique vegetation found in this area of Madagascar (a rare environment where the humid, dry and subarid biomes meet; see Buerki et al., 2013).

2. Material and methods

2.1. Sampling and DNA extraction

A list of specimens of *E. cynometroides* was established based on specimens deposited at G, K, MO and P (Table 1). To infer the spatial distribution of these specimens a map was constructed using the R packages 'RgoogleMaps' and 'rworldmap' (R Development Core Team, 2010). We have evidence suggesting that populations outside of Petriky are limited to very few individuals or even totally extinct due to anthropogenic habitat destruction and wood exploitation (see Section 2.2). In this study, we first investigate the phylogeography of *E. cynometroides* by sequencing the nuclear ITS region from herbarium specimens taken from individuals that are now extinct as well as recent collections, both extant and extinct (see introduction), obtained by the authors in Petriky. Subsequently, a DNA fingerprinting method (AFLP) is applied to assess the genetic variability of individuals in Petriky and to propose a conservation strategy. Herbarium specimens were not included in this latter analysis because their DNAs were too degraded for the AFLP technique to be suitable. Field-collected specimens were dried in silica-gel prior to DNA extraction, following the recommendations of Chase and Hills (1991), though some accessions were extracted from herbarium collections of varying ages. Extraction of genomic DNA followed the 2 × CTAB protocol (Doyle and Doyle, 1987), with the following