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Editorial
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Journal of Mesoamerican Biology
Special Issue: Biodiversity of the Cordillera del Merendón
Volume 1 (1), June, 2021

Editorial UNAH
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# Editor

It is my privilege to present the inaugural issue of the Journal of Mesoamerican Biology, a new and independent journal published by the Instituto de Investigación en Ciencias Biologicas y Ambientales del Norte de Honduras with the support of the Universidad Nacional Autónoma de Honduras en Valle de Sula. The regional, open-access model of the journal will provide an outlet that spans the breadth and depth of biodiversity research and topics, with an editorial and topical focus on the megadiverse countries of Central America.

With editors and peer-reviewers that are active specialists and practitioners in the region, Journal of Mesoamerican Biology provides a new and exciting forum for the dissemination of research results to an international audience. Articles are published in English, in order to maximize the international accessibility of the journal, and also included an enhanced Spanish-language abstract that summarizes each section of the paper. Submissions from authors who are not fluent in English are encouraged, and the editorial board is committed to facilitating the publication of works by Spanish speakers for maximum impact and availability on a global scale.

In the first issue, we present a collection of papers focused on the Merendón Mountains of northwestern Honduras, a hotspot of endemic biodiversity that includes the internationally renowned Cusuco National Park. These mountains are a Key Biodiversity Area that are recognized by the IUCN and Alliance for Zero Extinction for their unique and irreplaceable biota, many species of which endemic and globally endangered. While our knowledge of the Merendón biota is relatively well-developed for a few groups, such as birds, amphibians, and reptiles, for many taxonomic groups we have barely scratched the surface. We hope this special issue will begin to remedy that lack of information and point the way forward for future research.

The issues begins with an extensive and outstanding introduction, "A review of the ecological value of Cusuco National Park: an urgent call for conservation action in a highly threatened Mesoamerican cloud forest," which sets the stage for the rest of the issue and indeed for future research and conservation initiatives in the Merendón. This opening treatise is followed by articles on bat assemblages and their ectoparasites; the most comprehensive study ever presented on the rare and Critically Endangered giant treefrog *Ecnomiohyla salvaje*; a model-based assessment of high-value conservation areas for terrestrial mammals; and the first in a series of studies pioneering a fungal inventory of Cusuco National Park. To demonstrate the breadth of content being solicited for Journal of Mesoamerican Biology, there is a short paper reporting a distributional extension for a stream fish from the northern drainages of the Merendón.

I hope you enjoy the first issue of Journal of Mesoamerican Biology, and will join us in supporting and contributing to the growth of this important initiative.

JOSIAH TOWNSEND, PhD
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Centro Zamorano de Biodiversidad

Journal of Mesoamerican Biology
Special Issue: Biodiversity of the Cordillera del Merendón
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# A review of the ecological value of Cusuco National Park: an urgent call for conservation action in a highly threatened Mesoamerican cloud forest

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## Article info

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## Abstract

Cloud forests are amongst the most biologically unique, yet threatened, ecosystems in Mesoamerica. We summarize the ecological value and conservation status of a well-studied cloud forest site: Cusuco National Park (CNP), a 23,440 ha protected area in the Merendón mountains, northwest Honduras. We show CNP to have exceptional biodiversity; of 966 taxa identified to a species-level to date, 362 (37.5%) are Mesoamerican endemics, 67 are red-listed by the IUCN, and at least 49 are micro-endemics known only from the Merendón range. CNP also provides key ecosystem services including provision of drinking water and downstream flood mitigation, as well as carbon sequestration, with an estimated stock of 3.5 million megagrams of carbon in 2000. Despite its ecological importance, CNP faces multiple environmental threats and associated stresses, including deforestation (1,759 ha since 2000 equating to 7% of total forest area), poaching (7% loss of mammal relative abundance per year), amphibian declines due to chytridiomycosis (70% of species threatened or near-threatened), and climate change (a mean 2.6 °C increase in temperature and 112 mm decrease in rainfall by 2100). Despite conservation actions, including community ranger patrols, captive-breeding programmes, and ecotourism initiatives, environmental degradation of CNP continues. Further action is urgently required, including reinforcement and expansion of ranger programmes, greater stakeholder engagement, community education programmes, development of alternative livelihood projects, and legislative enforcement and prosecution. Without a thorough and rapid response to understand and mitigate illegal activities, the extirpation and extinction of species and the loss of vital ecosystem services are inevitable in the coming decades.

## Resumen extendido

**Antecedentes:** Los bosques nublados se encuentran entre los ecosistemas con mayor singularidad de biodiversidad, pero también entre los más amenazados en Mesoamérica. A pesar de esto, son raros los estudios de casos explícitos que resumen el valor ecológico particular de los bosques nublados, al igual que información sobre las amenazas que enfrentan y la efectividad de las intervenciones de conservación empleadas en ellos. Aquí resumimos el valor ecológico y el estado de conservación de un bosque nublado ampliamente estudiado: el Parque Nacional Cusuco, un área natural protegida de 23,440 ha ubicada en la cordillera del Merendón al noroeste de Honduras.

**Métodos:** Resumimos datos de más de cien publicaciones científicas, así como datos de varias fuentes inéditas, con el objetivo de proporcionar un resumen exhaustivo del valor ecológico del Parque Nacional Cusuco, las amenazas que enfrenta y el éxito de las intervenciones de conservación empleadas en el parque hasta la fecha. La mayoría de la información revisada proviene de los muestreos realizados por Operación Wallacea, que han sido efectuados estacionalmente en el parque en los últimos quince años. No obstante, se revisaron muchas otras fuentes de información, algunas de las cuales se remontan a la década de los ochenta.

**Resultados:** Demostramos que el Parque Nacional Cusuco tiene un valor excepcional de conservación, alberga por lo menos 327 especies de árboles y arbustos, 470 especies de vertebrados, y una diversidad de comunidades de artrópodos. Esto incluye 67 especies clasificadas globalmente como amenazadas y casi amenazadas, así como por lo menos 49 especies reconocidas como microendémicas para la región de la cordillera del Merendón. El parque proporciona servicios ecosistémicos claves, como la provisión de agua potable y la mitigación de inundaciones, el secuestro de carbono, con un stock estimado de 3,5 millones de megagramos de carbono generado en el año 2000.

A pesar de su gran importancia, el Parque Nacional Cusuco enfrenta múltiples peligros ambientales, incluyendo la deforestación (1,759 ha desde el año 2000, equivalente al 7% del área forestal total), caza ilegal (pérdida del 7% de la abundancia relativa de mamíferos por año), disminución de la población de anfibios por quitridiomicosis (70% de las especies catalogadas como amenazadas o casi amenazadas), y el cambio climático (un aumento promedio de 2.6 °C en la temperatura y una disminución de 112 mm en la precipitación durante el 2010). Las acciones de conservación que han sido implementadas incluyen patrullas comunitarias de guardaparques, programas de cría en cautividad *ex situ* e iniciativas de ecoturismo.

**Discusión:** A pesar de las acciones de conservación implementadas hasta la fecha, la degradación ambiental en el Parque Nacional Cusuco aún continúa. Se requieren medidas adicionales con urgencia, estas medidas incluyen incrementar el refuerzo y expansión de los programas de guardaparques, un mayor compromiso de las partes involucradas, programas de educación comunitaria, desarrollo de proyectos de estrategias alternativas de subsistencia para las comunidades, así como la aplicación y enjuiciamiento legislativo. Sin una respuesta rápida y exhaustiva para comprender y mitigar las actividades ilegales en el parque, serán inevitables las extirpaciones, extinciones de especies y la pérdida de servicios vitales del ecosistema en las próximas décadas.

## Introduction

Tropical montane cloud forests (‘bosque nublado’) are broadly defined as “tropical forests frequently covered in cloud or mist” (Stadtmuller 1987). They are geographically restricted, comprising between 2.5% (Cayuela *et al.* 2006a) and 14.2% (Mulligan 2010) of forest ecosystems worldwide, depending on the definition used. The term ‘cloud forest’ can apply specifically to ‘upper montane rain forest’ (as defined e.g. by Richards 1996), but, as in other sources, in this review we use the term in a broader sense which includes a range of broadleaved montane rain forest types. Cloud forests are ecologically unique and support a high diversity of flora and fauna (e.g. Leo 1995; Long 1995). They are centres of endemism for plants (Bubb *et al.* 2004), invertebrates (Anderson & Ashe 2000), and herpetofauna (Wilson & McCranie 2004) and provide habitats for 10% of all range-restricted bird species

(Stattersfield *et al.* 1998). As such, they are disproportionately well-represented within global biodiversity hotspots (Myers *et al.* 2000; Brooks *et al.* 2006) and "irreplaceable" protected areas (Le Saout *et al.* 2013). Cloud forests also provide a wide range of ecosystem services, including water catchment protection (water filtration and storage, and downstream flood mitigation), carbon sequestration and storage, nutrient cycling, and biodiversity mediated services such as adjacent crop pollination, soil aeration, waste removal, and pest biocontrol (Bubb *et al.* 2004; Martinez *et al.* 2009).

The Mesoamerican biodiversity hotspot (Myers *et al.* 2000) is particularly rich in cloud forests. Despite their well-recognized ecological importance (Rahbek *et al.* 2019), Mesoamerican cloud forests are critically threatened ecosystems, experiencing high rates of habitat loss (Cayuela *et al.* 2006b). For much of the early and mid-twentieth century the integrity of these forests remained relatively intact due to their inaccessible nature. However, in recent decades human population growth, mechanization of forestry activities, and improved accessibility have resulted in an increase in cloud forest deforestation and habitat degradation (Aldrich *et al.* 1997; Powell & Palminteri 2001; Hansen *et al.* 2020). They are also disproportionately threatened by the impacts of amphibian diseases (Scheele *et al.* 2019) and global climate change (Feeley *et al.* 2011; Fadrique *et al.* 2018).

Despite representing an urgent conservation priority (Bubb *et al.* 2004), detailed, cross-disciplinary case studies documenting specific sites remain scarce and geographically localized. While some sites in South America and southern Mesoamerica have been relatively well-studied (e.g. Leo 1995; Nadkarni & Wheelwright 2014), there is a paucity of research within northern Mesoamerican cloud forests. Most existing research from the region has focussed on Guatemala and Mexico (e.g. Renner *et al.* 2006; Martinez *et al.* 2009) and tend to be individual studies rather than the products of long-term multi-disciplinary research. Similarly, while conservation strategies to protect remaining areas of these northern Mesoamerican cloud forests have been proposed (e.g. Toledo-Aceves *et al.* 2011), few publications have evaluated the successes of these strategies.

Cusuco National Park (hereafter CNP or 'the Park'), located in the Departments of Cortés and Santa Bárbara, northwest Honduras (Fig. 1), is one of several protected areas within the Cordillera del Merendón (hereafter the Merendón range). To the best of the authors' knowledge, it is the most intensively studied site within these mountains, and possibly within northern Mesoamerican cloud forests generally. This is due to the long-term, annual, multi-taxa biodiversity monitoring programme run by the

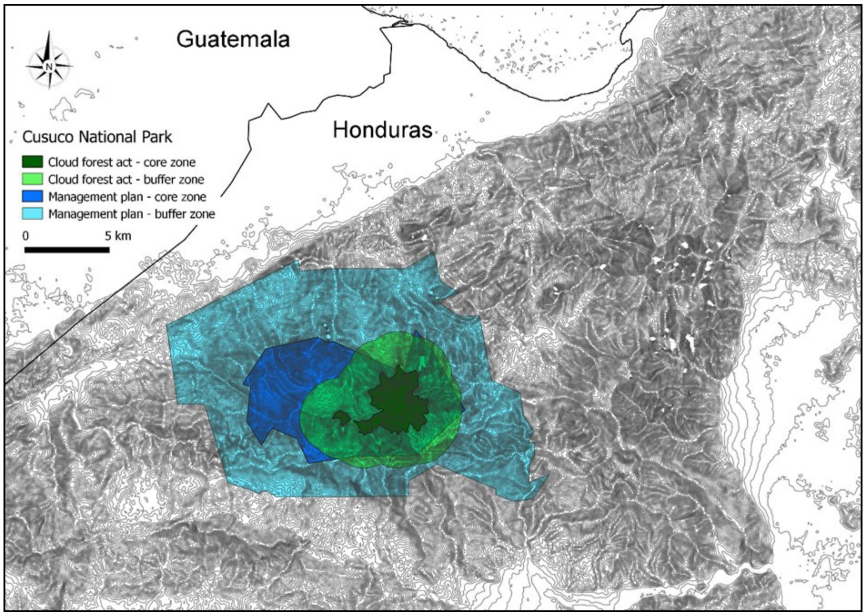

**Fig. 1.** Boundaries of Cusuco National Park, north-west Honduras, as defined by the Cloud Forest Act (1987) and the Park's Management Plan (1994).

UK-based expedition company Operation Wallacea (Opwall). This programme has been run in collaboration with the Instituto de Conservación Forestal (ICF) and academic partners worldwide. Surveys to date have consisted of an annual eight-week field season (June–August) between 2004 and 2019 (and occasionally interspersed with more specific fieldwork at other times of year), where most data have been collected along a network of permanent transects radiating from seven temporary forest camps (Fig. 2). While baseline biodiversity surveys and systematic monitoring efforts have been the primary focus of these expeditions, specific work has also been undertaken to quantify ecosystem services and identify and monitor environmental threats. This has allowed for the development of strategies for local conservation interventions. In addition to the Operation Wallacea programme, other organisations and individual scientists have also conducted research in CNP (particularly with regards to its herpetofauna which has been the subject of surveys since the 1980s – see McCranie & Wilson 1981). As such, CNP is well represented in the scientific literature, with 107 peer-reviewed scientific papers, three books/book chapters, and eight PhD theses all using primary data sourced from the Park published to date (see Supplementary Appendix SS1). These encompass fields as diverse as methodological survey and experimental design (Caras & Korine 2009; Martin *et al.* 2010; Erzberger *et al.* 2011; O'Callaghan & Kelly-Quinn 2012; Albergoni *et al.* 2016; Martin *et al.* 2017; McCravy *et al.* 2017), species descriptions (Lorence *et al.* 2015; Rodrigues *et al.* 2017), species inventories (Hoskins *et al.* 2018), ecology and behaviour (Jones *et al.* 2020a), comparative physiology (Jones *et al.* 2020b), population genetics (Asher 2009; Bun-

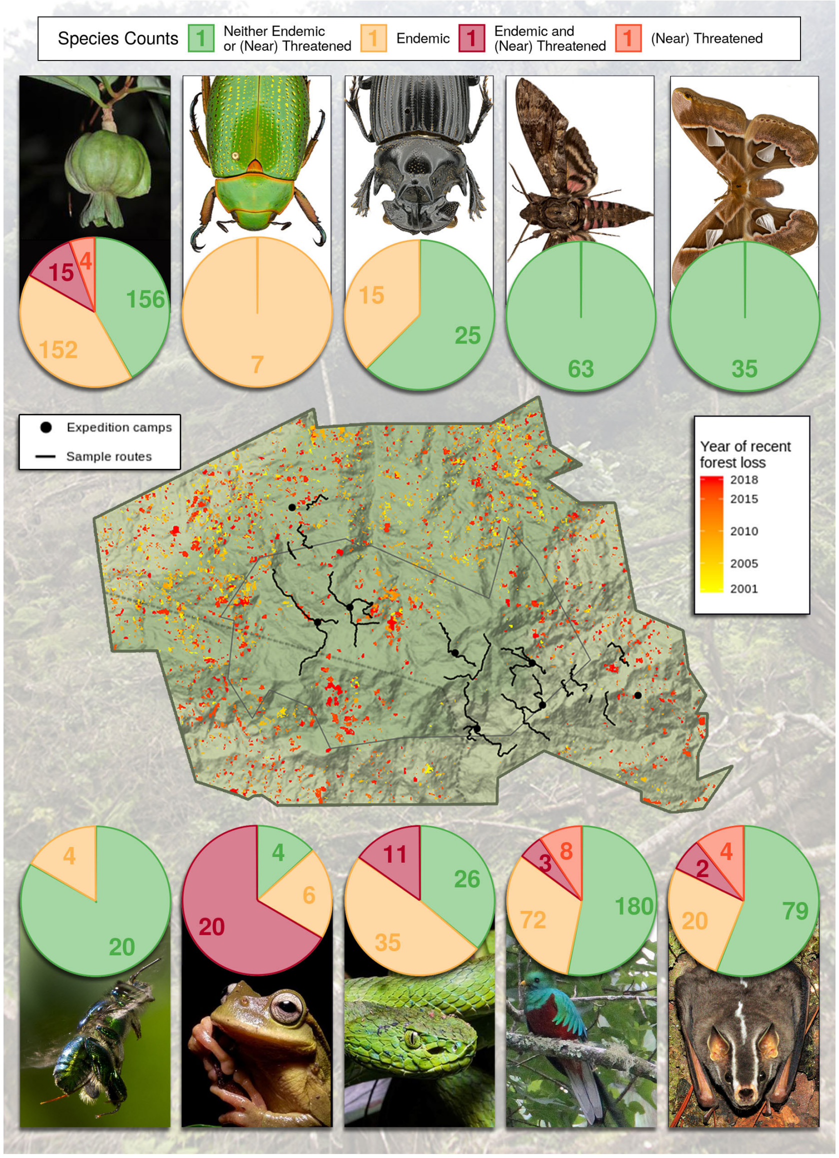

**Fig. 2.** A graphical overview of biodiversity, survey sites, and forest loss in Cusuco National Park. Centre: the scale of recent forest loss in CNP up to 2018 based on data from Hansen (2020). Areas not on the yellow-red spectrum have not undergone forest loss or were lost prior to 2000. The core zone of the park is denoted by the inner grey line, and the Operation Wallacea expedition sampling network shown by black points (camps) and lines (sample routes). Pie charts summarize data on taxa surveyed intensively in CNP. These are (top row): trees and shrubs, Chrysina jewel scarabs, Scarabaeinae dung beetles, Sphingid moths, Saturnid moths, (bottom row) Orchid bees, Amphibians, Reptiles, Birds, Mammals. Pie chart segments show numbers of species that are endemic to Mesoamerica (cream), threatened / near-threatened (red), endemic and threatened / near-threatened (magenta) and not endemic or threatened (green). Photo of E. imperialis copyright E. Littlefair, reproduced with permission. All other photos are copyright to authors or author institutions. Basemap by Stamen Design, under CC BY 3.0.

ting *et al.* 2016), applied conservation (McCann *et al.* 2012), species community responses to environmental change (Neate-Clegg *et al.* 2018), species-specific zoological studies for invertebrates (Stanbrook *et al.* 2017; Lonsdale & Brown 2019), fish (Olinger *et al.* 2016) and herpetofauna (Talley *et al.* 2005; Wilson *et al.* 2006; Hess *et al.* 2015; Clause & Brown 2017; Arrivillaga & Brown 2018; Brown & Arrivillaga 2018; Brown *et al.* 2018; Brown 2018, 2019, 2020; Brown *et al.* 2020; Lonsdale *et al.* 2020; Sasso *et al.* 2020), and contributions to global-scale macro-ecological studies (e.g. Cayuela *et al.* 2012; Crowther *et al.* 2015; Slik *et al.* 2015, 2018; Mendieta-Leiva *et al.* 2020).

We present a synthesis of research undertaken in CNP to date based on both annual field seasons (methodologies summarised in Gilroy *et al.* 2017) and findings from additional studies. First, we summarise the history, general ecology and ecosystems of CNP, and then specifically summarise and describe data on species richness, endemism, and threatened species for each major taxonomic group. We then assess ecosystem threats, the efficacy of conservation interventions, and finally, highlight further conservation actions necessary for the long-term preservation of CNP. We intend this review to represent a ‘call to arms’ for the better protection of CNP specifically, and Neotropical cloud forests generally.

# 1. Study site

CNP occupies the central portion of the Sierra Omoa, one of several smaller mountain chains that together comprise the Merendón range. The Park spans an elevational range of 500–2,242 m a.s.l (ICF 2015) (Fig. 1) and was established after the passing of the Cloud Forest Act (Act 87-1987) as part of the Sistema Nacional de Áreas Protegidas de Honduras (SINAPH) (Bonta 2005; Martin & Blackburn 2009). It was principally created to protect the watershed that supplies the second-largest city in Honduras, San Pedro Sula (population ~700,000), and the densely populated Sula Valley (Townsend & Wilson 2008). Similar watershed protection outcomes were also the main reason for the establishment of most other cloud forest national parks during this period (Cruz 1993).

The Park is adjacent to three other protected areas, including two forest regions protected under Act 53-1959 (Zona Forestal No2) and Act 210-1985, and a Water Protection Zone (Act 46-1990). The Park originally consisted of a core zone delineated as all terrain >1,800 m a.s.l and a surrounding 2 km buffer zone (Fig. 1). In 1991, the management responsibilities of CNP were passed from the Dirección General de Recursos Naturales Renovables (RENARE) to the Corporación Hondureña de Desarrollo Forestal (COHDEFOR). Following this, in 1994 a park management plan was published by COHDEFOR covering 23,440 ha which recommended an extension of the core zone to 7,690 ha, within which permanent settlements and any activities involving extraction of forestry resources were not permitted. The plan also advocated an extension of the buffer zone to 15,750 ha, within which some limited land use was permitted (Fig. 1). However, these proposed management plan boundaries have never been officially recognised by the Honduran government, and thus some uncertainty and controversy remain over the precise position of the Park’s boundaries. Land use zonation is further complicated by the disputed ownership of some land parcels within the Park (H. Hoskins *pers. obvs.*).

# 2. Ecological significance

## 2.1. Environmental conditions and vegetation types

The Merendón range was formed by a Palaeozoic granitic intrusion into volcanic ash sediments (Williams 2006). This granite is only exposed in riverbeds above 1,450 m a.s.l; elsewhere the surface geology is dominated by strata of gneiss and schist (Williams 2006). The terrain is rugged, with steep slopes, narrow ridges and deep valleys. Soils are strongly acidic throughout forested areas, with pH ranging from 3.0 to 4.5 in the top 5 cm (F. Brearley, *unpubl. data*). Total annual precipitation is *ca.* 3,000 mm with 45% of rainfall falling in the wettest months between October and December (Fundación Ecologista 1994). Mean day time temperatures in summer (June–July) range from 21 °C at 1,150 m a.s.l (range: 18 °C – 23.5 °C) to 15 °C at 2,200 m a.s.l (13 °C – 16.5 °C) (Jones 2020), with adiabatic lapse rates of 5–6 ºC/1000 m (typical of tropical mountains e.g. Freeman 2015).

The upper and middle elevational ranges of the Park (*c.* 1300 m – 2242 m a.s.l) are largely covered with closed canopy forest, much of which has experienced little anthropogenic disturbance, although patches of deforestation have begun to appear in recent years and are increasing annually. There are also extensive areas of secondary forest at various stages of successional development, mostly below 1,300 m a.s.l. Older stands of secondary forest are the result of historical commercial logging, which occurred from the 1950s to the 1980s. Younger stands occur partly due to more recent illegal deforestation and disturbance, and partly due to storm damage (Batke & Kelly 2015).

Most of the forest is dominated by a heterogenous community of broadleaved evergreen trees, with no single dominant species. From approximately 1,300–1,800 m a.s.l, in the Park's core zone, relatively intact lower montane rain forest predominates, interspersed with patches of secondary forest. The best-developed stands contain such characteristic lowland tropical elements as custard-apple family (Annonaceae) and strangler figs (*Ficus* spp.). Gaps in the forest canopy are quickly colonised by the fast-growing pioneer 'guarumo' or trumpet tree (*Cecropia peltata*). Characteristic trees across a range of elevations include oaks (*Quercus* – at least nine species), a large number of species in the laurel family (Lauraceae), and sweet gum (*Liquidambar styraciflua*), which is one of the few deciduous species and one of the tallest-growing trees in CNP, exceeding 60m in some locations. The forest understorey is largely dominated by tree-ferns, dwarf palms, and locally bamboos, along with a wide diversity of shrubs and smaller trees.

Areas of pine forest occur in CNP, particularly on the drier eastern slopes, and are notably less diverse than the broadleaved forest. Pine forest canopy is dominated by *Pinus maximinoi* (Maximino's pine, found in both primary and secondary forest across a range of elevations) and *P. tecunumanii* (Schwerdtfeger's pine, largely in secondary forest at lower elevations). Pines, oaks, and sweet gum all belong to the 'northern' biogeographical elements in the forest flora that are approaching their southerly range limits in Honduras.

With increasing elevation, the cooler temperatures and higher rainfall bring about a transition to upper montane rain forest ('true' cloud forest in a narrower sense) at approximately 1,800 m a.s.l. Community composition of trees here alters, but the most striking change is in the increasingly luxuriant growth of epiphytes. This vegetation zone is characterised by thick epiphytic growths of mosses, liverworts, ferns, bromeliads, and orchids. At altitudes >2,000 m a.s.l, on exposed mountain peaks and ridge tops, elfin forest ('*bosque enano*') occurs. Vegetation here is of low stature, comprising a densely interwoven canopy of small trees and shrubs, and with epiphytes growing right down to ground level. The topsoil is a spongy humus, the result of lower decomposition rates combined with intense leaching.

Concerning terminology: '*bosque enano*' (elfin forest) is widely used to refer to dwarf, moist, high-altitude forest/scrub. However, Mejía-Valdivieso (2001) differentiates between '*bosque musgoso*' (mossy forest)/'*bosque hepática'* ('hepatic forest'), which he records from the Cerro Jilinco area of CNP, and an even more stunted vegetation type that he denotes as true '*bosque enano*/dwarf forest', and records only from the Sierra Agalta.

A high abundance and diversity of epiphytes – plants which start life perched on a tree trunk or branch – is a characteristic feature of cloud forests (Bubb *et al.* 2004). Vascular epiphytes are very common throughout CNP (Batke *et al.* 2016), with the highest diversity being found in mid- and upper-elevation forest. These include holoepiphytes (which have no contact with the ground at any part of their life-cycle) and hemiepiphytes (which develop on another plant and send aerial roots towards the soil, allowing some to become large shrubs or even trees) (Moffett 2000). Holoepiphytes in CNP include orchids, ferns, and bromeliads, with a single tree able to host as many as 37 species (S. Batke, *unpubl. data*). Bromeliads are particularly conspicuous, with genera such as *Werauhia*, *Catopsis*, *Vriesea* and *Tillandsia* containing an 'aerial pond' or phytotelma (plural phytotelmata) at the centre of each leaf rosette; the associated fauna of which is of special interest to zoologists (see below). Hemiepiphytes also occur throughout, *Clusia* spp. being the most plentiful. The second major group of 'hangers-on' are the climbers *sensu lato* – plants which start life at ground level and then climb up the tree towards light. These may be subdivided into climbers *sensu stricto*, in which the plant remains rooted at the base and is hence stationary throughout its lifetime, and nomadic vines, which send out adventitious roots as they grow, allowing the plant as a whole to shift position in the course of its lifetime (Moffett 2000). In CNP, nomadic vines in the arum family (Araceae) are plentiful, notably the genera *Philodendron* and *Monstera*. The final group are the mistletoes which, unlike true epiphytes, are hemiparasitic, extracting water and dissolved nutrients from the xylem of the host tree.

The buffer zone, which surrounds the core zone in all directions at lower elevations, is composed of a mosaic of secondary forest, cropland, coffee plantations, and pasture for cattle, and includes 12 villages with a combined population of 3,082 (Honduran 2014 census data - https://www.ine.gob.hn/V3/).

The most abundant aquatic habitats in CNP are its network of rivers and streams. The Park has very few standing waterbodies. Most of these are artificial ponds in the vicinity of villages in the buffer zone, including small ponds maintained for fish cultivation. Besides rivers, the most common aquatic habitats are phytotelmata and water-filled tree holes.

## 2.2. Biodiversity

A compilation of survey data from Opwall (following methodologies summarized in Gilroy *et al.* 2017), along with other previously published data, indicates that 966 taxa have been identified to species-level in CNP to date, including 362 Mesoamerican endemics (37.5% of all species) and 67 threatened or near threatened species (including nine Critically Endangered species), with a further three species being listed as Data Deficient (Table 2). A total of 49 species (six plants, 21 arthropods, 22 vertebrates) are micro-endemics known only from the Merendón range (Table 3). We summarize key findings relating to diversity, endemism, and threat status of different taxa visually in Fig. 2. as well as in the sections below:

### Plants

The flora of CNP is diverse and supports many rare and little-known species. A total of 913 vascular plant species have been recorded to at least a morphospecies level from forested areas >500 m a.s.l. Of these, 49.4% are trees and shrubs, 22.1% epiphytes, 17.2% terrestrial herbs, 9.9% climbers and 1.4% mistletoes. Of the total, to date 78% have been identified to species-level, 15.4% to genus, 4.6% to family and 2% only as 'morphospecies' (D. Kelly & S. Batke, *unpubl. data*).

Taxonomic inventory work completed to date has focussed on the trees and shrubs, which constitute the 'framework' of the forest ecosystem. Of the 327 trees and shrubs identified to species-level, 167 (51.1%) are endemic to the Mesoamerican biodiversity hotspot (Table 1) and 19 (5.8%) are IUCN-listed as threatened or near threatened (Table 2). The list of micro-endemics includes three trees, two shrubs (dwarf palms; Hodel *et al.* 1995), and one herb (Table 3). Four of these species are newly described: *Hondurodendron urceolatum* ('Honduras Tree', Ulloa Ulloa *et al.* 2010), *Sommera cusucoana* ('Cusuco Coffee Tree', family Rubiaceae; Lorence *et al.* 2015), *Styrax paulhousei* ('Paul House's Snowbell Tree', family Styracaceae; Fritsch *et al.* 2018) and *Calathea carolineae* ('Caroline's calathea', family Marantaceae; Kennedy 2012). The discovery of *Hondurodendron* is particularly remarkable. A monotypic genus, it appears to be a palaeoendemic, its evolutionary isolation likely dating back to the period when the land that is now northern Honduras was an outpost at the southernmost tip of the North American continent (Montes *et al.* 2015). Three of these new species (*H. urceolatum*, *S. cusucoana, S. paulhousei*) were highlighted as being of high conservation concern in their initial descriptions, although to date all remain unassessed by the IUCN. *S. cusucoana* and *C. carolineae* are known solely from a small area in the west of CNP. Given the very small extent of known occurrence for these species, along with alarming deforestation trends in western CNP (see below), it is likely that all these species warrant Endangered or Critically Endangered status.

Surveys in CNP have also made a substantial number of additions to the Honduran flora; these involve major extensions to the known range of some species such as the fern *Serpocaulon lasiopus* (Batke & Hill 2013).

The low number of non-native species in the CNP flora, at least within the well-studied core zone, is striking – and a matter for favourable comment. Only one non-native tree species was recorded as naturalized: *Syzygium jambos* (Rose-apple, family Myrtaceae). A native of south-east Asia, it is widely cultivated in the tropics as a fruit-tree, but in many locations it has become a damaging invasive (Burman *et al.* 2017). Two non-native herbaceous species grown as ornamental garden plants in the region are naturalized in more disturbed forest within CNP: *Crocosmia* x *crocosmiiflora* (Montbretia, family Iridaceae) and *Impatiens walleriana* (Busy Lizzie, family Balsaminaceae).

The bryophyte flora of CNP has received only cursory investigation. Fifteen mosses have been identified (eleven to species level) and ten liverworts (four to species level) (Fundación Ecologista 1994; D.L. Kelly *unpubl. data*). In addition, some ecological studies carried out in the Park have used bryophyte cover as a proxy for microclimate conditions (Batke *et al.* 2015). At lower elevations, bryophytes are plentiful but generally not conspicuous. Even in the pine forests, where bryophyte cover is generally low, hummocks of the bog-moss *Sphagnum meridense* are locally frequent. The bryophyte flora increases in luxuriance and diversity with increasing elevation. In upper montane forest and elfin forest – where the humidity is more or less permanently close to saturation – trunks, branches and logs are swathed by a sward of leafy liverworts, mosses, filmy ferns, lichens and other epiphytes. This sward includes species of *Bazzania*, *Ceratolejeunea, Cheilolejeunea, Herbertus*, and *Lepidozia* — all liverwort genera that are characteristic of neotropical montane rain forest. Another feature of the upper montane zone is the pendant life form: bryophytes that hang down from tree trunks and branches, forming ‘streamers’ up to 20-40 cm long. This life form is represented in CNP by the mosses *Phyllogonium fulgens*, *Pilotrichella flexilis* and *Isodrepanium lentulum* and the leafy liverwort *Frullania convoluta*.

Ecological studies, particularly related to canopy epiphytes in CNP, have shown that the community structure is particularly influenced by differences in elevation, which correlates with changing vapour pressure deficit and historical wind disturbance from hurricanes (Batke & Kelly 2015). The impact of historical high energy weather events in CNP (e.g. hurricane Mitch in 1998) is most pronounced on south-facing ridges, as confirmed by visible tree impact assessments (Batke & Kelly 2014) and a hurricane impact model (Batke *et al.* 2014).

## TABLE 1. Endemic and threatened or near-threatened species in focal taxonomic groups in Cusuco National Park

| Group | # species | # endemics (%) | # IUCN threatened (%) |
|---|---|---|---|
| Trees and shrubs | 327 | 167 (49.0) | 19 (5.8) |
| Jewel scarabs | 7 | 7 (100) | 0 |
| Dung beetles | 40 | 15 (37.5) | 0 |
| Orchid bees | 24 | 4 (16.8) | 0 |
| Sphingid moths | 63 | N/A | 0 |
| Saturniid moths | 35 | N/A | 0 |
| Amphibians | 30 | 26 (93.3) | 20 (70) |
| Reptiles | 72 | 46 (63.9) | 11 (15.3) |
| Birds | 263 | 75 (28.5) | 11 (4.8) |
| Mammals | 105 | 22 (21.0) | 6 (5.7) |
| TOTAL | 966 | 362 (37.5) | 67 (7.1) |

**Table 1.** Endemic and threatened or near-threatened species in focal taxonomic groups in Cusuco National Park. Endemism refers to species restricted to the Mesoamerican biodiversity hotspot following Myers et al. (2000). Endemism status of moths is in the process of being assessed. Threat status follows IUCN (2020).

TABLE 2. Threatened, near-threatened and data -deficient species occurring in Cusuco National Park.

| Class | Order | Common name | *Scientific name* | IUCN status | Trend |
|---|---|---|---|---|---|
| Plantae | Pinales | Schwerdtfeger's pine* | *Pinus tecunumanii* | VU | ↓ |
| | Magnoliales | Cochrane's magnolia* | *Magnolia cochranei* | EN | ? |
| | | Orejuela* | *Cymbopetalum mayanum* | EN | ? |
| | Laurales | Cafecillo* | *Mollinedia butleriana* | CR | ? |
| | | Aguacate bajo* | *Persea donnell-smithii* | VU | ↓ |
| | Saxifragales | Montón* | *Molinadendron hondurense* | CR | ? |
| | Malpighiales | Meados de burro* | *Gloeospermum boreale* | CR | ? |
| | Fagales | Honduras walnut* | *Alfaroa hondurensis* | VU | ? |
| | | Roble de costa* | *Quercus insignis* | EN | ? |
| | | Skinner's oak * | *Quercus skinneri* | VU | ? |
| | Sapindales | West Indian cedar | *Cedrela odorata* | VU | ↓ |
| | Cornales | Disc-flowered dogwood* | *Cornus disciflora* | VU | ? |
| | Apiales | Mano de león* | *Oreopanax echinops* | VU | ? |
| | | Mano de león* | *Oreopanax sanderianus* | VU | ? |
| | Ericales | Cuya* | *Parathesis vulgata* | EN | ? |
| | Arecales | Oblong parlour palm* | *Chamaedorea oblongata* | VU | ↓ |
| | Lamiales | Azulito* | *Vitex cooperi* | EN | ? |
| | Caryophyllales | Guatuzo* | *Neea acuminatissima* | EN | ? |
| | Myrtales | Guayabo* | *Terminalia bucidoides* | EN | ? |

| | | | | | |
|---|---|---|---|---|---|
| Amphibia | Anura | Robber frog sp. # | *Craugastor charadra* | VU | ? |
| | | Coffee rain frog # | *Craugastor coffeus* | CR | ↓ |
| | | Robber frog sp. # | *Craugastor cyanochthebius* | EN | ↓ |
| | | Robber frog sp. * | *Craugastor laevissimus* | EN | ↓ |
| | | Broad-headed rainfrog * | *Craugastor laticeps* | NT | ? |
| | | Miles robber frog # | *Craugastor milesi* | CR | ? |
| | | Robber frog sp. * | *Craugastor rostralis* | VU | ↔ |
| | | Copan brook frog # | *Duellmanohyla soralia* | EN | ? |
| | | Copan tree frog # | *Ecnomiohyla salvaje* | EN | ↓ |
| | | Ranita de bromelia pequeña # | *Bromeliohyla melacaena* | EN | ↓ |
| | | Copan Stream Frog * | *Ptychohyla hypomykter* | VU | ↓ |
| | | Honduras spike-thumb frog # | *Plectrohyla dasypus* | CR | ↓ |
| | | Exquisite spike-thumb frog # | *Plectrohyla exquisita* | CR | ↓ |
| | Urodela | Salamander sp. # | *Bolitoglossa conanti* | VU | ↓ |
| | | Salamander sp. # | *Bolitoglossa diaphora* | EN | ↓ |
| | | Giant palm salamander * | *Bolitoglossa dofleini* | NT | ↓ |
| | | Dunn's mushroomtongue salamander # | *Bolitoglossa dunni* | EN | ? |
| | | Cortes salamander # | *Cryptotriton nasalis* | EN | ? |
| | | Salamander sp. # | *Nototriton brodiei* | EN | ? |
| | | Worm salamander sp. # | *Oedipina tomasi* | CR | ↓ |

| | | | | | |
|---|---|---|---|---|---|
| Reptilia | Squamata | Mountain lesser galliwasp # | *Diploglossus montanus* | EN | ↓ |
| | | Anole sp. # | *Anolis amplisquamosus* | CR | ↓ |
| | | Cusuco anole # | *Anolis cusuco* | EN | ↔ |
| | | Stadelman’s worm snake* | *Amerotyphlops stadelmani* | VU | ? |
| | | Cusuco earth snake # | *Geophis nephodrymus* | VU | ↓ |
| | | Espinal’s coffee snake * | *Ninia espinali* | NT | ↓ |
| | | Snake sp. # | *Omoadiphas aurula* | VU | ↓ |
| | | Graceful brown snake sp.# | *Rhadinella pegosalyta* | VU | ↓ |
| | | Monte cristi graceful brown snake * | *Rhadinella montecristi* | VU | ↓ |
| | | Cloud forest parrot snake* | *Leptophis modestus* | VU | ↓ |
| | | March’s palm pit viper * | *Bothriechis marchi* | EN | ↓ |
| | | | | | |
| Aves | Tinamiformes | Great tinamou | *Tinamus major* | NT | ↓ |
| | Galliformes | Highland guan * | *Penelopina nigra* | VU | ↓ |
| | | Great curassow | *Crax rubra* | VU | ↓ |
| | Accipitriformes | Ornate hawk-eagle | *Spizaetus ornatus* | NT | ↓ |
| | Apodiformes | Black swift | *Cypseloides niger* | VU | ↓ |
| | Trogoniformes | Resplendent quetzal* | *Pharomachrus moccino* | NT | ↓ |
| | Coraciiformes | Keel-billed motmot * | *Electron carinatum* | VU | ↓ |
| | Passeriformes | Olive-sided flycatcher | *Contopus cooperi* | NT | ↓ |
| | | Wood thrush | *Hylocichla mustelina* | NT | ↓ |
| | | Golden-winged warbler | *Vermivora chrysoptera* | NT | ↓ |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
|  |  | Golden-cheeked warbler | *Setophaga chrysoparia* | EN | ↓ |
|  |  |  |  |  |  |
| Mammalia | Cingulata | Northern naked-tailed armadillo | *Cabassous centralis* | DD | ? |
|  | Eulipotyphla | Omoa broad-clawed shrew# | *Cryptotis mccarthyi* | DD | ? |
|  | Cetartiodactyla | Central American red brocket | *Mazama temama* | DD | ? |
|  | Perissodactyla | Baird's tapir | *Tapirus bairdii* | EN | ↓ |
|  | Chiroptera | Van Gelder's bat * | *Bauerus dubiaquercus* | NT | ? |
|  |  | Eastern pipistrelle | *Perimyotis subflavus* | VU | ↓ |
|  | Carnivora | Jaguar | *Panthera onca* | NT | ↓ |
|  |  | Margay | *Leopardus wiedii* | NT | ↓ |
|  | Primates | Mantled Howler Monkey | *Alouatta palliata* | VU | ↓ |
|  |  |  |  | Total species | 70 |

**Table 2.** Threatened, near-threatened and data -deficient species occurring in Cusuco National Park. Status follows IUCN (2020). ↓ = a declining species trend, ↔ = stable trends and ? = unknown trends, * are endemic to the Mesoamerican biodiversity hotspot (Myers et al. 2000), # are micro-endemics known only from the Merendón Mountain range and immediately adjacent areas. DD = Data Deficient, NT = Near Threatened, VU = Vulnerable, EN = Endangered and CR = Critically Endangered. For Plantae, only trees and shrubs were assessed.

TABLE 3. Micro-endemics occurring in Cusuco National Park which are restricted to the Merendón range and immediately adjacent areas. † indicates IUCN (2020) threatened status.

| Class | Order | Common name | *Scientific name* |
|---|---|---|---|
| Magnoliopsida | Gentianales | Cusuco coffee tree | *Sommera cusucoana* |
| | Santalales | Honduras tree | *Hondurodendron urceolatum* |
| | Ericales | Paul House's snowbell tree | *Styrax paulhousei* |
| Liliopsida | Arecales | Forked-leaf parlour palm | *Chamaedorea frondosa* |
| | | Molina's parlour palm | *Chamaedorea molinana* |
| | Zingiberales | Caroline's calathea | *Calathea carolineae* |
| Clitellata | Haplotaxida | Potworm sp. | *Bryodrilus hondurensis* |
| | | Potworm sp. | *Bryodrilus hondurensis* |
| Hexanauplia | Cyclopoida | Copepod sp. | *Olmeccyclops hondo* |
| | Harpacticoida | Copepod sp. | *Moraria catracha* |
| | | Copepod sp. | *Moraria cusuca* |
| Ostracoda | Podocopida | Seed-shrimp sp. | *Elpidium merendonense* |
| | | | |
| Arachnidae | Opiliones | Cosmetid sp. | *Eucynorta rooneyi* |
| | Trombidiformes | Watermite sp. | *Hydrodroma moralesi* |
| | | Watermite sp. | *Flabellifrontipoda triscutata* |
| | | Watermite sp. | *Monatractides angelae* |
| | | Watermite sp. | *Pseudotorrenticola espinasseae* |

| | | | |
|---|---|---|---|
| | | Watermite sp. | *Atractides jenniferae* |
| | | Watermite sp. | *Recifella cusucoensis* |
| | | | |
| Insecta | Coleoptera | Longhorn beetle sp. | *Derobrachus cusucoensis* |
| | | Jewel scarab sp. | *Chrysina cusuquensis* |
| | | Jewel scarab sp. | *Chyysina pastori* |
| | | Jewel scarab sp. | *Chrysina porioni* |
| | | Plant Beetle sp. | *Electribius llamae* |
| | Diptera | Moth-fly sp. | *Moruseodina cusucoensis* |
| | | Chironomid sp. | *Polypedilum panacu* |
| | | | |
| Amphibia | Anura | Robber frog sp. † | *Craugastor charadra* |
| | | Coffee rain frog † | *Craugastor coffeus* |
| | | Robber Frog sp. † | *Craugastor cyanochthebius* |
| | | Miles robber frog † | *Craugastor milesi* |
| | | Copan brook frog † | *Duellmanohyla soralia* |
| | | Copan tree frog † | *Ecnomiohyla salvaje* |
| | | Ranita de bromelia pequeña. † | *Bromeliohyla melacaena* |
| | | Honduras spike-thumb frog † | *Plectrohyla dasypus* |
| | | Exquisite spike-thumb frog † | *Plectrohyla exquisita* |
| | Urodela | Salamander sp. † | *Bolitoglossa conanti* |
| | | Salamander sp. † | *Bolitoglossa diaphora* |
| | | Dunn’s mushroomtongue salamander † | *Bolitoglossa dunni* |
| | | Cortes salamander † | *Cryptotriton nasalis* |

|  |  |  |  |
|---|---|---|---|
|  |  | Salamander sp. † | *Nototriton brodiei* |
|  |  | Worm salamander sp. † | *Oedipina tomasi* |
|  |  |  |  |
| Reptilia | Squamata | Mountain lesser galliwasp † | *Diploglossus montanus* |
|  |  | Anole sp. † | *Anolis amplisquamosus* |
|  |  | Cusuco anole † | *Anolis cusuco* |
|  |  | Cusuco earth snake † | *Geophis nephodrymus* |
|  |  | Snake sp. † | *Omoadiphas aurula* |
|  |  | Graceful brown snake sp. † | *Rhadinella pegosalyta* |
|  |  |  |  |
| Mammalia | Eulipotyphla | Omoa broad-clawed shrew | *Cryptotis mccarthyi* |
|  |  | Cruz's Long-tailed Shrew | *Sorex cruzi* |
|  |  |  | *Total species - 49* |

**Table 3.** Micro-endemics occurring in Cusuco National Park which are restricted to the Merendón range and immediately adjacent areas. † indicates IUCN (2020) threatened status. True numbers of micro-endemic plants and invertebrates are likely to be considerably higher than displayed due to lack of study.

**Fungi.** The first opportunistic fungi survey was completed in CNP in 2019. A total of 93 fungi have thus far been identified to order, 66 to genus and 25 to species-level. Of the fungi examined, 38 were identified as Agaricales (gilled mushrooms), followed by 11 of both Helotiales (cup fungi) and Hypocreales (well-known as insect pathogens). Of the fungi identified to genus level, ten were *Marasmius*, seven *Xylaria*, and five *Amanita*. ITS sequences have been generated and analysed for 18 collections. A BLAST search in NCBI GenBank for ten sequences resulted in 89.05–97.60% identity hits, implying that these collections either represent undescribed species or species that have not yet been sequenced. Given that other studies have highlighted high degrees of fungal endemism in cloud forest ecosystems (Smith *et al.* 2013; Bandala *et al.* 2016; Del Olmo-Ruiz *et al.* 2017), it is likely that there are multiple undescribed species of fungi in CNP. Based on the 2019 fieldwork alone, one new species – a crust-like fungus *Trechispora hondurensis* (*Trechisporales*) – has been described (Haelewaters *et al.* 2020) and additional undescribed species are expected in the following genera: *Chlorociboria*, *Ionomidotis* (Helotiales), *Cyathus*, *Gymnopus*, *Mycena*, *Pterula* (Agaricales), *Mycocitrus* (Hypocreales), and *Xylaria* (Xylariales). In the coming years, a formal fungal ATBI project will be initiated within CNP, which will collect and describe specimens from above-ground ephemeral fruiting bodies of non-lichenized fungi (*sensu* Haelewaters *et al.* 2018). In addition, we also plan to collect fungi associated with arthropods including *Hypocreales* and *Laboulbeniales*; these groups remaining very poorly studied within tropical forest ecosystems (Araújo & Hughes 2016; Blackwell *et al.* 2020).

**Arthropods.** Comprehensive taxonomic assessment of arthropods is incredibly challenging in tropical forest ecosystems (Basset *et al.* 2012). Major surveys, for which sampling has been sufficient to confidently estimate total species richness, have focused on Scarabaeinae dung beetles, *Chrysina* jewel scarabs, saturnid and sphingid moths, and orchid bees (Euglossini). Sampling of these taxa has been largely a result of the availability of appropriate expertise, as well as the ecosystem importance of the Scarabaeinae. Surveys completed to date have detected 40 species of Scarabaeinae (T. Creedy *et al.*, *unpubl. data*), seven species of *Chrysina* (Jocque *et al.* 2013a; Monzón Sierra & Hawks 2020), 24 species of Euglossini (including two new country records) (McCravy *et al.* 2016), 63 species of Sphingidae (including multiple new country records; Vanhove *et al.* 2012) and 35 species of Saturnidae (S. Waters, *unpubl. data*) (Table 1). All *Chrysina* species, four Euglossini and at least 15 Scarabaeinae are Mesoamerican endemics; endemism status of moth species is in the process of determination. No threatened species from any of these groups have been detected in CNP to date.

Small scale surveys on other groups have also been completed. These have detected 112 ant species (Antweb, 2020), 30 as-yet-undescribed species of Curculionidae and Staphylinidae beetles, all of which are thought to be Park endemics (Anderson & Ashe 2000), and eight species of Cosmetidae harvestmen (Damron 2020) including at least one that is new to science (Damron *et al.* 2018), along with at least 10 morphospecies of other Opiliones families (Damron 2014). Further *ad hoc* collection in CNP has yielded numerous new species discoveries including a large longhorn beetle (Santos-Silva *et al.* 2018), an elateroid beetle (Gimmel & Bocakova 2015), several leaf litter copepods (Fiers & Jocque 2013), and a new mantid genus (Rodrigues *et al.* 2017).

Long-term studies of tank bromeliad systems (Jocque *et al.* 2010) have revealed a high diversity of arthropod species, with high endemism within the chironomids (Mendes *et al.* 2011), oligochaetes (Schmelz *et al.* 2015), and psychodids (Bravo *et al.* 2014). Aquatic bromeliads in CNP have up to seven species of passively dispersed crustaceans, including at least one endemic seed-shrimp (Pinto & Jocque 2013). This is the highest regional record for phytotelmata biodiversity worldwide (Jocque *et al.* 2013b). Research in CNP has shown phytotelmata invertebrate community diversity to be positively related to bromeliad size (Jocque & Field 2014). Due to their inherent ephemerality, predation and resource use are the main factors structuring phytotelmata communities (Petermann *et al.* 2015; Cereghino *et al.* 2018).

Research into communities of lotic invertebrates in the streams and rivers of CNP indicate that such communities are overwhelmingly dominated by insects (98.5%), and that at least 136 species of 87 families occur, including 15 beetle families, 14 caddisfly families, 14 fly families (O'Callaghan & Kelly-Quinn 2017), and six water mite species (Wiles 2005). Community composition in different river systems is driven by a variety of factors, with elevation and water pH being particularly important (O'Callaghan & Kelly-Quinn 2017).

These data undoubtedly represent just a small fraction of true arthropod diversity in CNP, as evidenced by DNA-based surveys. For example, more than 8,000 operational taxonomic units (OTUs, presumptive species) were uncovered among the 50,000 arthropod specimens individually analysed using DNA barcoding from understorey malaise trap collections across 750 trap-days (D'Souza & Hebert 2018). In the canopy, more than 900 arthropod OTUs were detected in a single tree using metabarcoding (Creedy *et al.* 2019), and over 7,000 from modest sampling of two tree species from four localities in the core zone (Creedy 2018). These results reveal high levels of compositional and phylogenetic turnover in both understory and canopy communities, driven by the elevational gradient and high habitat heterogeneity across the core zone of the park. Overall, these DNA-based studies provide an insight into the high arthropod diversity supported in CNP, a large portion of which await discovery.

**Herpetofauna.** CNP is acknowledged as an internationally important site for threatened amphibians and reptiles (Le Saout *et al.* 2013; AZE 2018; BirdLife International 2020). A total of 102 species have been found here, included 72 mesoamerican endemics (70.6% of all species) and 31 threatened or near-threatened species (30.4% of all species) (Table 1). These totals include eight micro-endemics known only from CNP, all of which are IUCN listed. These are the Critically Endangered *Anolis amplisquamosus* (McCranie *et al.* 1992), *Plectrohyla dasypus* (McCranie & Wilson 1981), *Plectrohyla exquisita* (McCranie & Wilson 1998), and *Oedipina tomasi* (McCranie 2006a); the Endangered *Bolitoglossa diaphora* (McCranie & Wilson 1995) and *Bromeliohyla melacaena* (McCranie & Castañeda 2006); and the Vulnerable *Rhadinaea pegosalyta* (McCranie 2006b) and *Geophis nephodrymus* (Townsend & Wilson 2006). The Park is also a key stronghold for three further Critically Endangered and Endangered species that are endemic to the Merendón range: *Craugastor coffeus* (McCranie & Köhler 1999; Kolby 2009), *Craugastor milesi* (Kolby & McCranie 2009), and *Ecnomiohyla salvaje* (Solis *et al.* 2017). Ongoing additions of species to the Park's herpetofauna checklist include several snakes (Townsend *et al.* 2005a,b), anoles (Townsend & Plenderleith 2005), salamanders (Townsend *et al.* 2006; Kolby *et al.* 2009), and frogs (Kolby & McCranie 2009). Later additions have generally come from less-studied ecosystem frontiers, such as the tree canopy (Solis *et al.* 2017).

**Birds.** A total of 263 bird species have been definitively recorded in CNP, comprising 46 different families (S. Jones, *unpubl. data*). At least 24 additional species have been reported, but the authenticity of these records requires confirmation.

Of the recorded bird species, six are classified by the IUCN (2020) as Near Threatened, four as Vulnerable, and one as Endangered (Table 2). A number of the typical highland species of CNP are northern Mesoamerican endemics, such as the Highland Guan (*Penelopina nigra*), White-faced Quail-Dove (*Zentrygon albifacies*), Green-throated Mountain-Gem (*Lampornis viridipallens*), Emerald-chinned Hummingbird (*Abeillia abeillei*), and Rufous-browed Wren *Troglodytes rufociliatus* (Fagan & Komar 2016). These species, along with several threatened taxa (e.g. Resplendent Quetzal *Pharomachrus moccino* and Keel-billed Motmot *Electron carinatum*; Table 2), are dependent on intact montane forest, and their abundance in CNP declines away from the core zone of the Park (Martin & Blackburn 2009). Avian richness and diversity patterns inherently decline with increasing elevation (McCain 2009) but, since the initiation of standardized monitoring in 2007, the species composition of CNP has seen upslope shifts in many forest-dependent species, such that species composition at higher elevations has seen increasing influences by species communities typical of lower elevations (Neate-Clegg *et al.* 2018; Neate-Clegg *et al.* 2021).

Owing to seasonal timing of our annual fieldwork, the Park's resident avifauna is substantially known (e.g. Martin *et al.* 2016). However, CNP is also a key site for Nearctic migrants (e.g. Komar *et al.* 2011). Approximately 46 migratory species have been recorded in CNP, including three Near Threatened species (Olive-sided Flycatcher *Contopus cooperi*, Golden-winged Warbler *Vermivora chrysoptera*, and Wood Thrush *Hylocichla mustelina*), and one Endangered species (Golden-cheeked Warbler *Setophaga chrysoparia*) (Table 2). The Park is a key site for Golden-cheeked Warbler, the wintering distribution of which is restricted almost entirely to the highland Pine-Oak (*Pinus-Quercus* spp.) forests of Guatemala and Honduras (Groce *et al.* 2010). In contrast to many resident species, however, many of these migrants are capable of utilizing, to a degree, degraded forest and non-forest habitats (e.g. Rappole *et al.* 1999; Wunderle & Latta 2000), such as shade-grown coffee plantations in the buffer zone on the east side of CNP. Feather samples from 11 species of migrant warblers collected during winter fieldwork have also been contributed to the 'genoscape' project (www.birdgenoscape.org), a transnational study examining the breeding origins of declining Nearctic migrant songbirds.

## Mammals

There are 46 non-volant mammal species recorded from 26 families in CNP (Table 1); 43 of these are inventoried in Hoskins *et al.* (2018), with the remaining three species being a newly-described species of shrew (*Sorex cruzi*) (Andino-Madrid *et al.* 2020), an as-yet unidentified water mouse (see below), and the Central American Woolly Opossum (*Caluromys derbianus*). A total of 59 bat species from six families have been recorded to date (Medina-Van Berkum *et al.* 2020). Of the 105 mammal species that occur, 22 are Mesoamerican endemics and six are of conservation concern: the Endangered Baird's tapir (*Tapirus bairdii*), the Vulnerable mantled howler monkey (*Alouatta palliata*), and eastern pipistrelle (*Perimyotis subflavus*) and the Near Threatened margay (*Leopardus wiedii*), jaguar (*Panthera onca*), and Van Gelder's bat (*Bauerus dubiaquercus*).

A further three species in CNP are classified as Data Deficient (Table 2). One of these, the Omoa broad-clawed shrew (*Cryptotis mccarthyi*) is one of two micro-endemic mammals (along with the newly described *Sorex cruzi*) restricted entirely to the Merendón range. Given their small geographic range, it is likely both these species will be listed as threatened when sufficient information is available - a trend that is common for range-restricted Data Deficient species (Bland *et al.* 2015). Water mouse (*Rheomys* spp.) specimens have been captured along high-elevation streams - the first records of this genus within Honduras. While they are still to be fully identified, preliminary analyses indicate that their gross external phenotype and skull morphology differ significantly from Thomas's water mouse (*R. thomasi*) and Goldman's water mouse (*R. raptor*), found respectively to the north in Mexico and Guatemala and south-west in El Salvador. Further, it appears the Honduran specimens are more distinct from those species than they are from one another, suggesting a new species, subspecies, or race; molecular genetic analyses are ongoing (N. Reid, *unpubl. data*). Bat surveys have yielded numerous important range extensions, most notably the funnel-eared bat *Natalus lanatus*, which is the second record of this species in Honduras (Medina-Van Berkum *et al.* 2020).

## International ecological significance

Biodiversity surveys demonstrate CNP to possess high richness and high endemism, and to support large numbers of globally threatened species in a relatively small area. CNP represents just 0.02% of the total extent of the Mesoamerican biodiversity hotspot yet supports at least 3.8% of plant species, 16.4% of vertebrate species, and 14.6% of regionally endemic vertebrates found in this hotspot, following species totals given in Myers *et al.* (2000).

The true biodiversity of CNP is inevitably even greater than that summarised above, given the seasonal nature of most fieldwork (e.g. Nearctic migratory birds are likely underrepresented), the many micro-habitats that remain under-surveyed (e.g. the canopy) and the many species that are likely cryptic, both in terms of their ecology and their genetics. Irrespective of these gaps in surveying, the international importance of CNP is shown by its listing as the 123rd most 'irreplaceable' protected area globally (as well as the 48th for threatened taxa and 25th for threatened amphibians) (Le Saout *et al.* 2013) and its designation as a Key Biodiversity Area (BirdLife International 2020) and an Alliance for Zero Extinction site (AZE 2018). The conservation value of CNP additionally extends beyond these biodiversity statistics; it is also important for ecological connectivity on a landscape scale (DeClerck *et al.* 2010). In particular, it represents an important section of the Central American jaguar corridor, facilitating movements between populations in Honduras and populations in Guatemala and Belize (Wultsch *et al.* 2016; Petracca *et al.* 2017). Jaguars remain extremely rare within the Park. Despite a seven-year camera trapping survey carried out throughout the Merendón range by Panthera, only a single photographic record of a jaguar was captured in CNP: a young adult male that remained in the Park´s core zone for over one month in 2016 and was never recorded in the area again (F. Castañeda *et al.*, *unpubl. data*). We speculate this could have been a dispersing individual traveling between the established jaguar populations at the Nombre de Dios mountain range in Honduras and the Selva Maya in Guatemala. This points to the importance of small protected areas such as CNP as stepping-stones along the jaguar corridor, providing resting and feeding grounds for traveling individuals, and sustaining landscape-scale connectivity for this large carnivore.

Diversity and endemism in CNP are driven by several factors, including the overlapping of Nearctic and Neotropical species that are characteristic of Mesoamerica (the 'Great American Biotic Interchange' Stehli & Webb 1985; Morrone 2010), and the elevational turnovers and biogeographical isolation inherent to tropical montane forests (Körner & Spehn 2002) which drives both endemism and high beta diversity (Shmida & Wilson 1985). Moreover, CNP has both a Caribbean and a Pacific/inland-facing slope, each possessing different microclimates (Martin *et al.* 2016).

While the ecosystems of CNP are evidently of international significance, it is important to note that high biodiversity and endemism are the rule rather than the exception for tropical cloud forests (e.g. Bruijnzeel *et al.* 2011). Accurately comparing biodiversity between Mesoamerican cloud forests is invariably difficult owing to disparities in survey effort, widely differing sizes and altitudinal ranges, and the geographical attributes of remaining cloud forest fragments (Schuster & Cano 2006). That being said, a comparison of woody plant diversity in CNP with four comparable protected cloud forest areas in Honduras (Table 4), for example, shows broadly similar species richness among four of the five sites (the lower diversity for the Texiguat-El Paraíso site reflects its narrower altitudinal range). Basic comparisons of other taxa from other Mesoamerican cloud forest sites show similar trends. For example, Monte Verde in Costa Rica, perhaps the only other Mesoamerican cloud forest with a comparable intensity and duration of vertebrate monitoring and surveillance effort, has somewhat greater mammal (155 vs 105 species) and herpetofauna (161 vs 102 species) diversity, and substantially greater bird diversity (500 vs 263 species) than CNP (Bermúdez *et al.* 2005; Nadkarni & Wheelwright 2014). Such differences may come about due to Monteverde being larger, more heterogenous (albeit covering a smaller elevational range), further south along the latitudinal diversity gradient, and more intensively studied, with surveys dating back to the 1970s (Nadkarni & Wheelwright 2014). Comparisons of Scarabaeinae dung beetle fauna, meanwhile, suggest CNP to have higher diversity than cloud forest sites in Veracruz, Mexico (Pineda *et al.* 2005), although this is likely due, at least in part, to a sampling bias. The euglossine bee community in CNP, on the other hand, is less speciose than reported from a Panamanian study site (Ackerman & Roubik 2012); this is most probably a reflection of the smaller elevational range studied in CNP.

Overall, available data indicates that Cusuco is not necessarily exceptional with regards to the number of species found within its borders. It is unique, however, with respect to the community of species it supports. A comparison of the tree and shrub floras of CNP with the four other Honduran cloud forest Protected Areas mentioned above shows CNP to be

TABLE 4. Comparisons of woody plant diversity (trees and shrubs identified to a species level) between Cusuco National Park and four other protected Honduran cloud forest sites: Sierra de Agalta, Pico Bonito, El Paraíso, and Uyuca.

| Protected Area | Area ($km^2$) | Altitude (m) | Distance from CNP (km) | # species identified to species level | # species shared with CNP | Sørensen coefficient of similarity between floras | # IUCN-listed threatened species | # IUCN-listed threatened species from that area only |
|---|---|---|---|---|---|---|---|---|
| Cusuco National Park (CNP) | 234.4 | 500-2242 | NA | 327 | NA | NA | 19 | 10 |
| Sierra de Agalta | 207.9 | 1800-2354 | 300 | 223 | 82 | 0.30 | 8 | 3 |
| Pico Bonito | 564.3 | ~100-2435 | 172 | 291 | 94 | 0.30 | 15 | 6 |
| Texiguat, El Paraiso | ~1,900 | 1244-1648 | 270 | 96 | 23 | 0.11 | 2 | 0 |
| Uyuca (combined datasets) | 5.79 | 800-2008 | 242 | 247 | 65 | 0.23 | 7 | 1 |

Table 4. Comparisons of woody plant diversity (trees and shrubs identified to a species level) between Cusuco National Park and four other protected Honduran cloud forest sites: Sierra de Agalta, Pico Bonito, El Paraiso, and Uyuca. Diversity values in these other reserves are based on Pfeifer (1960), House et al. (2006), Carbajal Vásquez (2010), and unpublished data from Zamorano herbarium. Sørensen coefficient values quantify similarity of woody plant community composition between Cusuco National Park and the other four sites. Assessment of threat status (Vulnerable, Endangered or Critically Endangered) is taken from IUCN (2020).

distinct from all the other sites, as evidenced by the low Sørensen coefficient values (Table 4). Each site also supports different threatened species: only one of a total of 29 IUCN-listed species was recorded from all five sites (Table 4). It seems clear that each Mesoamerican cloud forest site represents its own unique 'island in the sky'. However, the comparison of tree and shrub data shows that sites can be far from equal in overall conservation value. Some had large numbers of threatened species: CNP topped the list, with 19 threatened tree & shrub species, of which ten were not recorded from any of the other sites. This was independent of site size (e.g. Pico Bonito National Park was second to CNP despite being more than twice the size) (Table 4).

## 2.3. Ecosystem services

In addition to its biodiversity value, CNP also provides a wide range of the ecosystem services. Perhaps most significantly, the Park forms part of the watershed for the city of San Pedro Sula and parts of the Greater Sula Valley, providing clean water as well as flood and soil erosion prevention services to *ca.* 800,000 people (www.citypopulation.de). Indeed, safeguarding this part of the Merendón water catchment was the principal reason CNP was initially designated a protected area.

A further key service provided by CNP is carbon sequestration. Cloud forests, due to their small extent, fragmented nature, and steep topography, do not generally store as much carbon as intact lowland tropical forest ecosystems (e.g. Martin *et al.* 2015). Nonetheless, they are regionally still important carbon reservoirs (Leija-Loredo *et al.* 2018). Neotropical montane forests contain on average 123.5 MgC/ha (megagrams of carbon per hectare) aboveground (Spracklen & Righelato, 2014) with a further 25% (30.9 MgC/ha) belowground (Cairns *et al.* 1997), yielding a total standing stock of 154.4 MgC/ha. In 2000, CNP was estimated to be 94% forested with 22,790 ha of extant forest (Hoskins 2019). Derived from these figures, a preliminary baseline estimate might suggest the Parks' ecosystems sequestered 3.5 million MgC in 2000.

## 3. Environmental threats, stresses, and contributing factors

Despite its high biological importance, CNP faces a range of environmental threats, driven by various contributing factors, which have led to stresses such as habitat loss, severe defaunation, and release of greenhouse gases (Salafsky *et al.* 2008). The four most acute threats facing CNP are deforestation, poaching, disease, and climate change (Fig. 3). We discuss the extent and consequences of each of these threats in turn.

### 3.1. Deforestation and habitat degradation

Threats driving habitat loss within cloud forest ecosystems include conversion to agricultural land and livestock grazing, timber extraction, and infrastructural (road and village) development (Aldrich *et al.* 1997; Bubb

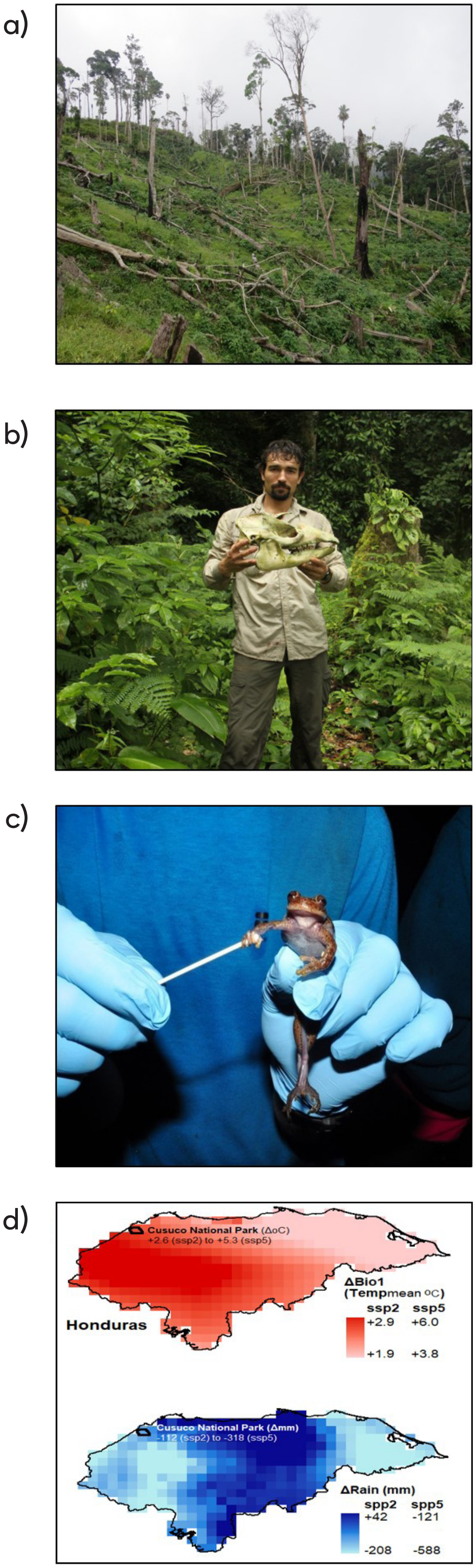

**Fig. 3.** The four principal environmental threats in Cusuco National Park: deforestation, poaching, disease, and climate change. A) Recent deforestation photographed in July 2019 in the western section of the Park's core zone. B) The skull of a recently poached Baird's tapir *(Tapirus bairdii)* found in CNP. C) A frog being swabbed for PCR detection of *Batrachochytrium dendrobatidis* (chytrid fungus) the causative agent of Chytridiomycosis. D) Predicted climate change throughout Honduras highlighting Cusuco National Park in mean temperature in °C and total rainfall in mm between the averages for 1970–2000 and 2081–2100, using the 6th Model for Interdisciplinary Research on Climate (MIROC6) assuming Shared Socioeconomic Pathways SSP2 (the 'Middle of the Road' scenario) and SSP5 ('Fossil-fueled Development' scenario) using 100 grid cells [data extracted from https://worldclim.org].

*et al.* 2004). These have a range of negative impacts which have been associated with tropical deforestation generally (e.g. Harris *et al.* 2012; Alroy 2017), although many of these impacts are exacerbated in cloud forests because they tend to be spatially restricted and highly fragmented (Cayuela *et al.* 2006a; Toledo-Aceves *et al.* 2011).

Honduras possesses one of the highest deforestation rates of any Central American country (Magrin *et al.* 2014), and one of the worst rates globally for deforestation in protected areas (Hansen *et al.* 2020). Analysis of satellite imagery (following Hansen *et al.* 2013) suggests CNP lost >7% (ca. 1,759 ha) of forest from 2000 to 2017, with an average annual loss of 103 ± 99 ha (Hoskins 2019). Annual deforestation rates were highly variable during this period, but worse in recent years (e.g. 2016 and 2017), suggesting this threat is accelerating. Adopting average aboveground carbon stock values from Spracklen & Righelato (2014), we estimate that since 2000 around 217,237 MgC have been released into the atmosphere due to forest loss in CNP (approx. 6.2% of the total carbon store). This is a conservative estimate and does not include releases from belowground carbon stores because the rate of their loss to the atmosphere remains unknown, as does the extent to which replacement agricultural soils act as net carbon sources. The cumulative trend in forest loss has been increasing at a nearly linear rate, with models predicting a doubling of the area deforested (and atmospheric carbon enrichment) over the next 20 years if no effective conservation actions are implemented (Hoskins 2019).

Contributing factors driving habitat conversion in CNP are primarily agricultural, involving the planting of both subsistence crops such as maize and beans, cash-crops such as coffee (the most significant crop on deforested land, at least where favourable conditions occur) and cardamom, and cattle farming. Small-scale marijuana cultivation also occurs in more remote clearings within the Park. The actors associated with the contributing factors of this deforestation range from local subsistence farmers to wealthy individuals financing land clearance and subsequent agricultural development. While reasons for deforestation are broadly similar throughout CNP, patterns of habitat loss are not (Figure 2). In recent years deforestation has been much more pronounced in the northern and western sections of the Park (Fig. 3a), rather than the south and east. The contributing factors driving this disparity are complex. More remote regions of CNP (particularly the western side of the park) are further from central authority in San Pedro Sula and lack a permanent military presence and regular community ranger patrols (see section 4.1), both of which are present in the east, and thus illegal land clearance is more common. Specifically, deforestation on the northern and western slopes of the park may be seen as of less concern because run-off flows directly into the Caribbean Sea rather than into the heavily populated Sula valley (which relies on forest cover for clean water and flood protection). At present, pristine, closed canopy forest in the less protected northwest of CNP's buffer zone, particularly in areas of flatter topography and in proximity to other recently deforested areas, are at greatest risk of forest loss (Figure 2) (Hoskins 2019).

As a result of land conversion, nutrient and sediment run-off have been shown to act as a stressor on the richness and abundance of freshwater macroinvertebrates in small streams in CNP (O'Callaghan *et al.* 2015), although the catchment-scale impacts of this run-off remain unevaluated..

## 3.2. Poaching

Unsustainable hunting of large vertebrates has been identified as a key threat to biodiversity globally (Maxwell *et al.* 2016) and has been particularly severe in the neotropics (WWF 2018). Additionally, cloud forest ecosystems may be disproportionately impacted by hunting. This is because populations of large-bodied species here tend to be biogeographically isolated in small, genetically depauperate populations with limited immigration to help re-colonization after local extirpation (Brown & Kodric-Brown 1977). Moreover, small habitat patches are more easily accessible to poachers (Bubb *et al.* 2004). The collapse of large-bodied vertebrate populations in cloud forest ecosystems is a matter of great concern, as these populations are associated with key ecosystem services such as seed dispersal and nutrient cycling (Jones & Safi 2011). Forest carbon sequestration rates have also been associated with mammalian species richness and abundance (Osuri *et al*., 2016).

Community ranger patrols run between 2015 and 2020 (see section 4.1) have logged 147 events corresponding to evidence of poaching in CNP. Most of these events involved the identification of hunting blinds (tapescos in Spanish); a wooden platform constructed high in the trees and used specially to target paca (*Cuniculus paca*). Other poaching evidence includes bullet shells, hunting camp remains, and carcasses.

Analysis of mammalian tracks and signs on standardised transects surveyed annually from 2010 to 2018, adjusted for survey effort, suggests the relative abundance of all terrestrial mammals in CNP has declined by 7% per year on average, with most rapid declines in the Park's more accessible and less rigorously protected buffer zone (Hoskins *et al*. 2020). The Park was once regarded as a Honduran stronghold of the Baird's tapir (McCann *et al*. 2012; McCann 2015), yet recent work suggests it is approaching extirpation from CNP due to poaching (Fig. 3b) and habitat degradation (Hoskins *et al*. 2020). It might be expected that large hunted species (e.g. deer) would exhibit more rapid declines than unhunted species (e.g. wild cats, mustelids etc.). However, even the latter have declined by 6% per year (Hoskins *et al*. 2020), suggesting other threats, such as deforestation and disturbance, are of comparable importance as stressors of population change. Such is the rapidity of observed population declines that it is estimated that populations of many large-bodied mammals in CNP could become functionally extinct as early as the mid-2020s (Hoskins *et al*. 2020).

## 3.3. Amphibian diseases

Chytridiomycosis is an infectious disease of amphibians caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*). It has caused population declines in over 500 species, with nearly 100 species driven to extinction, and as such is responsible for greater biodiversity loss than any other disease in recorded history (Scheele *et al*. 2019; Fisher & Garner 2020).

*Bd* has been reported from numerous localities in Honduras (Puschendorf *et al*. 2006; Gutsche *et al*. 2015), including CNP where it was first detected in 2007 (Kolby *et al*. 2010). Retrospective analysis of archived museum material confirmed the presence of chytrid in CNP as early as 1996 (Kolby & Padgett-Flohr 2009), and thus it is likely this pathogen arrived in the region during the early 1990s, if not earlier. Five *Bd* prevalence datasets derived from amphibian swabbing (Fig. 3c) have been analysed. Using PCR analyses, Kolby *et al*. (2009) reported an overall *Bd* prevalence rate of 44.4% from a sample of 257 amphibians of 16 species swabbed in 2007, with certain life-stage groups exhibiting much higher rates (96.2% in juveniles of the Critically Endangered *Plectrohyla dasypus*, N = 27). Clake (2015), using qPCR analyses, reported an overall *Bd* prevalence rate of 19.5% from 848 individuals of four species swabbed between 2011–2014, with highest prevalence rates (31%) being reported in *Ptychohyla hypomykter* (N = 102). Kolby *et al*. (2015b) reported an overall prevalence of 88.5% (N = 52) among juvenile frogs of four species threatened with extinction using qPCR analysis of skin swabs. Blooi *et al*. (2017) reported a prevalence rate of 12.7% in stream-dwelling amphibians (N = 150) and 3.4% in bromeliad-dwelling amphibians (N = 116) from qPCR analysis of swabs collected in 2014 and 2015. A further collection of 80 swabs analysed using qPCR analyses in 2017 reported an overall *Bd* prevalence rate of 57.5%, rising to 64.7% in *P. dasypus* (N = 34) and 100% in *P. hypomykter* (N = 4) (C. Phipps, *unpubl. data*). These data show prevalence rates to be generally high, but variable between species and years. Contributing factors driving this are likely to be, respectively, species' ecology (Blooi *et al*. 2017) and annual variability in rainfall and water availability (Clake 2015; Ruggeri *et al*. 2018).

Samples of chytrid collected from CNP belong to at least two divergent chytrid lineages. Of 20 samples where the lineage could be confirmed, 18 were comprised of the globally distributed hypervirulent *Bd*GPL lineage, and the other two samples were found to be the *Bd*Cape lineage, apparently endemic to Africa (Byrne *et al*. 2019). These data are noteworthy as they represent the first known presence of *Bd*Cape in the western hemisphere. How *Bd*Cape was introduced to CNP is unclear and warrants further investigation. Regardless, the presence of *Bd*Cape in CNP is a cause for concern, because it presents the opportunity for the generation of highly virulent hybridized strains. Although *Bd* is most often clonal, the phenomenon of sexual recombination was confirmed in Brazil between *Bd*GPL and *Bd*Brazil (Schloegel *et al.* 2012), with the offspring expressing greater virulence towards native amphibians than either of the parental strains (Greenspan *et al.* 2018).

*Bd* is ubiquitous in CNP and has been detected in nearly all locations where extensive sampling has been completed and where suitable hosts were found. Detection of *Bd* in both rainwater (Kolby *et al.* 2015a) and on the surfaces of vegetation (Kolby *et al.* 2015b) shows how easily it can disperse across the landscape and be transmitted when susceptible amphibians encounter viable spores. A diverse range of amphibians have tested positive for infection, spanning genera that include stream, epiphyte, and canopy-dwelling specialists (Kolby *et al.* 2010; Blooi *et al.* 2017; Thorp *et al.* 2021), although more research is needed to establish whether they all develop the disease chytridiomycosis. Interestingly, results in CNP suggest that bromeliads may represent refugia for certain species as the prevalence rate reported in phytotelmic microhabitats by Blooi *et al.* (2017) was five times lower than in nearby stream habitats. A possible explanation could be due to the low pH of bromeliad water (Jocque & Kolby 2012) or the ability of micro-eukaryotes to ingest chytrid zoospores present in bromeliad tanks (Blooi *et al.* 2017). Interpretation of these findings should, however, be treated with caution, for two reasons. Firstly, while some studies have reported similar results (Lindquist *et al.* 2011), others have demonstrated high *Bd* spore loads and high rates of *Bd* infections within phytotelmata, both in lowland forests (McCracken *et al.* 2009) and cloud forests (Cossell & Lindquist 2009). Secondly, *Bd*-associated mortality rates of amphibians utilizing phytotelmic microhabitats in CNP – which can be very different from chytrid infection rates – have not yet been examined. Further research is therefore necessary before conclusions can be drawn, although it is encouraging to see lower amounts of *Bd* in these arboreal habitats.

Overall, chytridiomycosis represents a critical threat to amphibians throughout CNP, and it may also threaten other species. Amphibian population collapses have knock-on effects on their predators, notably amphibian-feeding snakes (Zipkin *et al.* 2020). In CNP this may impact on species such as the range-restricted Palm viper *Bothriechis marchi*, a frog-eating specialist. A critical avenue for further research is the quantification of temporal population fluxes among threatened amphibians, and the consequences for predator-prey dynamics.

## 3.4. Climate change

Cloud forests occupy narrow high-elevation climatic niches typified by high rainfall. As such, they are disproportionately impacted by global climate change (Williams *et al.* 2003). Global warming has been shown to cause upslope shifts in species ranges, where the combined effects of competition, shifting habitat boundaries, and shifts in climate envelopes cause mountain top extirpations; the 'escalator to extinction' scenario (Fadrique *et al.* 2018; Freeman *et al.* 2018).

Climate modelling forecasts CNP to be particularly at risk within Honduras, with a 2.6 ºC increase in mean annual temperature (from 23.0 ºC to 25.6 ºC) and a 112 mm decrease in total annual rainfall (from 1,785 mm to 1,673 mm) predicted by the end of the century (2081–2100) in comparison to historical average conditions (1970–2000) (Fig. 3d). These results are based on climate data downloaded from WorldClim (Fick & Hijmans 2017) and the MIROC6 climate model (Tatebe *et al.* 2019) and assume modest projections of global socioeconomic change (i.e. the Shared Socioeconomic Pathway 2, SSP2) (Riahi *et al.* 2017). The most extreme projections (SSP5) suggests that the mean temperature of the park could rise by 5.3 ºC with a 318 mm decrease in annual rainfall (Fig. 3d). This increase in temperature will increase evaporation which, together with the expected decreases in precipitation, is predicted to reduce water inflow into Honduran reservoirs by up to 20% (Maurer *et al.* 2009). Honduras has experienced increasingly frequent and prolonged droughts in recent decades (Rauscher *et al.* 2008) resulting in soil moisture deficits and failures of both subsistence and cash crops (Bunn *et al.* 2018; Calvo-Solano *et al.* 2018). Such stochastic events are likely to cause even more extreme peaks in future temperatures, and more impactful droughts. This is likely to have serious socio-economic consequences, with both climate change and ongoing deforestation likely to have negative impacts on the water provisioning capacity of the Park, as well as its ecological communities.

Montane specialist species are and will be particularly stressed by climate change, either as a direct result of warming on species' thermal physiology (see Polato *et al.* 2018) or as a result of the multiple drivers of habitat loss, interspecific competition, and shifting ecotones that favour lower-altitude generalist species (Jankowski *et al.* 2012). Arthropods inhabiting the rainforest canopy, unprotected by the buffering effect of overhead vegetation, are likely to be affected more acutely by climate change (Nakamura *et al.* 2017). Strong community partitioning and phylogenetic structure among canopy arthropods across small scales (<3 km) in elevational climatic zones in CNP (Creedy 2018) suggests this portion of the Park's fauna are particularly vulnerable to the 'escalator to extinction' effect (Freeman *et al.* 2018). Strong community partitioning patterns have also been observed in CNPs bird community (Jones *et al.* 2020a) and may be similarly vulnerable to this effect.

Upslope shifts in the bird communities of CNP over a ten year period from 2007–2016 have been documented, with elevational shifts consistent across all habitats and also only those in closed-canopy primary forest that had seen no land conversion over that period (Neate-Clegg *et al.* 2018; Neate-Clegg *et al.* 2021. These results suggest that elevational upslope shifts in the avian community are being driven primarily by climate change. At present this is the only quantitative assessment of the effects of climate change on species communities in the Park; analyses are currently underway utilising the strength of standardised and repeated cross-taxon study.

A final consequence of climate change may be an increase in the frequency and intensity of Caribbean hurricanes (Knutson *et al.* 2010) which may have long-lasting impacts on community composition and vegetation structure in Mesoamerican cloud forests (Tejeda Cruz & Sutherland 2005; Batke & Kelly 2015). Additionally, stronger and more frequent hurricanes may also destroy park infrastructure, making cloud forest protected areas more difficult to manage, and also damage livelihoods in communities surrounding these protected areas, making people more reliant on illegal resource extraction. Both these factors could indirectly threaten the integrity of Mesoamerican cloud forest ecosystems. At the time of writing, CNP had just experienced what is believed to be its most destructive hurricane season in decades, with a category 4 and a category 5 storm both causing widespread tree damage, landslides, and destruction of property. The short-term and long-term consequences of these hurricanes will be empirically assessed in future research seasons.

# 4. Conservation actions

The high biodiversity value of CNP, coupled with the urgency of the environmental threats and associated stresses it faces, have led to a range of conservation actions, which we detail in turn below.

## 4.1. Community ranger patrols

In August 2012 the Honduran Minister of Natural Resources and Environment directed military personnel to patrol CNP. These patrols used locally hired guides and were employed as a deterrent against the threats of illegal deforestation and poaching. These patrols were *ad hoc* and no data were collected on their efficacy, although some illegal activities were directly disrupted (F. Castaneda, *pers. comm.*). In August 2015, the Jaguares de Cusuco Community Ranger Team was formally established with financial support from Panthera and the Wallacea Trust, and this team have since been carrying out regular patrols in CNP. These rangers patrol using GPS devices, and collect data (e.g. direct observation of poachers, discovery of hunting platforms or poached carcasses) using SMART conservation software (https://smartconservationtools.org). At the time of writing, the SMART database included data on 425 patrols (representing a total of 6,108 km walked) completed between 2015 and 2020. These patrols were focussed towards the southern and eastern parts of the Park due to the increased risk of patrolling in the more remote western and northern sections of CNP. Vegetation in two recently deforested areas is regenerating with no further signs of deforestation after being patrolled intensively following initial detection, indicating the value of this conservation action as a deterrent.

Prior to the instigation of the community ranger programme, deforestation would often go undetected for long periods, giving loggers time to build permanent structures (fences, outbuildings, and even houses) on the cleared patches. Honduran law offers certain rights to squatters who have built infrastructure, even on illegally occupied ground. Early detection of deforestation events is therefore highly important, and the community ranger programme offers an effective way to achieve this.

Rangers also log illegal poaching and have recorded 147 incidences so far (see section 3.2.), mostly related to Least Concern species such as lowland paca (*Cuniculus paca*) and white-tailed deer (*Odocoileus virginianus*), although evidence of poaching Endangered species, including the Baird's tapir, has also been recorded. A passive acoustic monitoring survey was also started by Panthera in 2018, with support from the U.S. Fish and Wildlife Service, to monitor poaching activity indirectly. Percussion detection devices have been positioned in trees around CNP to record frequency, timing, and approximate location of gunshots fired in the Park. This acoustic survey is helping to quantify poaching activity in CNP and will play a central role in optimising anti-poaching patrolling calendars. Preliminary results indicate that poaching events are negatively correlated with the temporal and spatial distribution of patrols, as well as with the presence of the annual Operation Wallacea expedition season (F. Castañeda *et al.*, *unpubl. data*). The main limitation of the programme to date is its lack of coverage across the whole of CNP; a substantial issue given that the most extensive deforestation occurs in areas where at present it is difficult for rangers to operate.

## 4.2. *Ex-situ* captive breeding for reintroduction

*Ex-situ* conservation methods are often a highly feasible conservation action for amphibians given that they are generally relatively inexpensive to maintain, often breed quickly and in high numbers, and tend to cope physiologically and behaviourally with captive conditions better than larger-bodied taxa (Bloxam & Tonge 1995; Balmford *et al.* 1996). Proof of success for the captive breeding model for amphibians can be seen in the numerous species saved from extinction by *ex-situ* measures (e.g. Dreitz 2006; Lee *et al.* 2006). The number of amphibian species held *ex-situ* in the face of the unfolding amphibian extinction crisis is rapidly growing, both in zoos and smaller 'non-traditional' facilities such as private breeding centres (Biega *et al.* 2017). Facilities located in the country where the captively bred species originate are particularly desirable, given the opportunities for greater engagement with local stakeholders, prevention of disease transmission, better integration of *ex-situ* and *in-situ* strategies, and avoidance of entanglement with restrictive legislation on international movements of wildlife (Martin *et al.* 2014; Biega *et al.* 2017).

One such private breeding facility, the Honduras Amphibian Rescue and Conservation Centre (HARCC 2020), is being developed at Lancetilla Botanical Garden, approximately 70 km to the north-east of CNP. The goal of this centre is to perform head-start and reintroduction activities to prevent the chytrid-driven extinction of three of CNP's threatened and endemic amphibian species: *Plectrohyla dasypus*, *Plectrohyla exquisita*, and *Duellmanohyla soralia*. To help these populations persist in the wild, HARCC's main objective is to collect young frogs from CNP before they die from chytridiomycosis, care for them at the biosecure HARCC research facility, and then reintroduce them back into CNP as healthy adult frogs. A captive assurance programme will also be developed to maintain a breeding population of frogs at HARCC so that animals can continue to be released back into CNP even in the case of a sudden extinction event in the wild. To date, the necessary infrastructure for HARCC has been completed, but official permission has yet to be provided for the transfer of frogs from CNP to begin. These delays are cause for concern, given the severe stresses amphibians in CNP are facing, and the small window of time that may be available to develop conservation actions to protect them from extinction.

## 4.3. Ecotourism

Ecotourism has long been highlighted as a means to conserve biodiversity by placing an economic value on keeping ecosystems undisturbed (Boo 1990) and enhancing management capacity building for protected areas (Yu *et al*. 1997). It is often the case that locations which are hotspots for ecotourism are not correlated with sites experiencing high threat, nor those which support high concentrations of endemic species (Kruger 2005). However, CNP (which has both of these attributes) would, in theory at least, be well-placed to benefit from both domestic and international tourism based both on its conservation need and its proximity to San Pedro Sula and its international airport.

To date, tourism in CNP (and in most of Honduras) remains limited. The single biggest source of visitors to the Park is the Opwall research programme, which has brought an average of 350 students and 70 scientists to CNP yearly over the period 2004-2019. As well as paying park fees, these visitors provide seasonal employment for approximately 90 people from surrounding communities as guides, cooks, porters, and other logistical staff. The programme (which is only able to draw visitors due to CNP's exceptional biodiversity) thus generates important revenue for the Park authorities and provides income streams to local communities, albeit for a limited part of the year and for a limited selection of communities. There is also some circumstantial evidence to suggest that the presence of sizeable numbers of visitors in the forest during the Opwall season acts as an active deterrent to illegal activities in CNP. While many of the Opwall long-term survey plots have been deforested in the last 16 years, particularly in the western half of the Park, such deforestation seldom occurs in the June-August period while the expedition is running. Similarly, preliminary acoustic data indicate that poaching events diminish during the Opwall research season (see section 4.1).

As well as conspicuous benefits, it is important to note that visitors to CNP may also have unintended consequences. Camera trap data from CNP have shown that detection rates of hunted large-bodied mammals (deer, peccaries, etc) are highest further from forest camps, presumably due to these species avoiding human disturbance (Hoskins 2019). Camera trap detection of unhunted mammals (e.g. wild cats, mustelids etc.) is highest further from forest trails (which are maintained by annual clearing with a machete and are subject to high daily human traffic during the Opwall season). Care must thus be taken to manage the anthropogenic impacts of large visitor groups so as to minimise negative stresses on wildlife.

One of the biggest challenges in channelling the positive aspects of ecotourism to achieve conservation is ensuring involvement of as many communities as possible. Again, in this respect communities in the northern and western parts of CNP benefit less from ecotourism activities than elsewhere. While the Operation Wallacea programme is not large enough to achieve involvement in all of CNP's communities, finding means to extend other ecotourism ventures elsewhere would be a valuable step.

## 4.4. Urgent call for conservation and management actions

Despite the implementation of various conservation actions, illegal deforestation and poaching continue in CNP at an alarming rate, indicating that these interventions alone are insufficient to arrest these trends. Theoretically, the status of CNP as a formal protected area should by itself be sufficient to protect against deforestation and poaching threats, but in practice this is not so. Deforestation within protected areas is widespread in the Neotropics (e.g. Bonham *et al*. 2008) and is a particularly acute problem in Honduras (Hansen *et al*. 2020). Funding for CNP is extremely limited, and the capacity of official bodies to effectively monitor and prevent illegal activities is severely restricted. In some other Honduran protected areas co-management agreements between ICF and local communities have been developed to partially offset the lack of centralized funds supporting conservation actions (ICF 2010). However, at present CNP has no such arrangement. Conservation action in CNP is further complicated by the lack of clear park borders, resulting in ambiguity around the legality of some deforestation and hunting activities. Furthermore, arresting and prosecuting lawbreakers in national parks is potentially dangerous across much of Central America (as demonstrated in the difficulties of organizing community patrols in certain parts of the Park). As such, legislative enforcement and prosecution of illegal activities are somewhat limited in Honduras. This, coupled with the socioeconomic contributing factors of many of these threats, means that actions which entirely focus on prosecution or enforcement may not bring positive change to CNP.

A more holistic approach to conservation - that involves protection enforcement alongside sustainable resource management, while taking social and political contributing factors into account – may offer a more bespoke and positive way forward to resolving environmental issues in CNP (Redford *et al.* 2006). Efficient resolution of human-natural ecosystem conflicts is based on a thorough socio-economic and cultural understanding of the situation and typically requires a multi-dimensional approach, often involving education and awareness programmes, both of which require time. However, rates of habitat loss and poaching in CNP are such that little time remains to prevent irrevocable damage. An intensification of conservation actions is therefore urgently needed in the short term, coupled with a need for more long-term socio-economic strategies. We identify a series of urgent short-term and longer-term conservation actions which need to be implemented in order to safeguard the future of the Park. The successful implementation of these will reduce environmental stresses within CNP by targeting the threats that create these stresses.

## Urgent short-term conservation actions:

1) Achieve the compliance and enforcement of environmental regulations within CNP by:

> *a) Recognizing and communicating precise park borders.* Achieving official recognition of the extended delineation of CNP as defined by Corporación Hondureña de Desarrollo Forestal (COHDEFOR) in the park management plan published in 1994, with a subsequent clear communication of these borders (and the legal consequences of infringing them) delivered to surrounding communities, would remove ambiguity about the legality of deforestation and hunting within the area.

> *b) Expanding the spatial and temporal coverage of community ranger patrols.* Anecdotally, patrols appear to be a strong deterrent to poaching in the vicinity of Buenos Aires village on the eastern side of the Park. Most of the community rangers live in this village, and it is likely that a resident ranger presence based in other settlements would also discourage local poaching. A higher frequency of patrols throughout the year would also improve their effectiveness.

> *c) Improving government and military responses.* It is vital that authorities respond in a timely manner to information on illegal activities provided by the community rangers, and that the judiciary receive dedicated training to ensure that appropriate sanctions are handed out to the perpetrators of these crimes in accordance with Honduran law. The establishment of a permanent ICF presence within the Park would be a strong step towards facilitating swift responses to illegal activities in CNP.

2) Safeguard threatened species which are declining due to disease by:

> *a) Conducting species-specific rescue actions.* Given the number of threatened micro-endemics in CNP, species-specific conservation actions will play an important role in averting extinctions. A notable example is the proposed HARCC *ex-situ* conservation facility.

## Long-term conservation objectives:

1) Achieve long-term sustainable resource use in the communities surrounding CNP by:

> *a) Completing extensive social science surveys in communities surrounding the Park.* A fundamental first step in achieving long-term sustainable resource use in CNP is to develop a detailed understanding of the drivers and usership of resources and the economics thereof in the communities surrounding the Park. Social science data providing this information is vital

with respect to ensuring the success of the actions listed in points b,c,d and e below (Dayer *et al.* 2020).

*b) Developing alternative sustainable livelihoods for communities.* Providing communities currently dependent on income generated from illegal activities within CNP boundaries with alternative incomes equal or greater to those derived from such activities would, where feasible, reduce economic incentives to deforest and poach. Initiatives could include; nursery plantations and subsequent reforestation for internationally funded carbon sequestration projects; more efficient agriculture in the buffer zone; ecotourism; sustainable harvesting of forest resources; and development of value-added products placed in both domestic and international markets. Such projects would provide a better balance between biodiversity, the services provided by the ecosystem, and the economic well-being of the people who live in and around the Park (Kremen & Merenlander 2018).

*c) Establishing a co-management framework that boosts stakeholder participation in CNP.* This will allow members of local communities (often represented by local authorities such as water boards and town boards – 'patronatos'), non-governmental institutions, other civilians, and national government to work together for the management of CNP, and would enhance the effectiveness of many of the other conservation actions listed here.

*d) Developing education and training.* Such programmes would empower local communities with the skills, knowledge, and confidence to engage in sustainable local resource management and raise environmental awareness. These would seek to achieve a mentality change and would involve communications with all the communities in and around CNP.

*e) Boosting ecologically sensitive ecotourism.* Ecotourism, if correctly developed, could provide an important source of income for local communities and a revenue stream for the management of CNP, as well as raise awareness of its conservation value. Links with Honduran schools, universities and other key institutions are essential. There already seems to be a growing number of visitors from an increasingly affluent San Pedro Sula. Avitourism is likely to be an effective means of attracting international visitors; the rich bird community and numerous regional endemics are potentially a strong draw to what has become a lucrative international industry. It is vital for any tourism development in CNP to be conducted in a responsible manner so as to avoid negative impacts on wildlife. Better economic management of tourism in CNP is also necessary. For example, at present there is no effective means of collecting entry fees from tourists, but this could be rectified by placing manned toll booths at entry points to the Park or within the Parks communities.

2) Achieve a better long-term understanding of ecological trends and threat impacts in CNP by:

*a) Expanding biodiversity and ecosystem research and monitoring.* While the ecology of certain species and ecosystems in CNP are relatively well-known, many remain unstudied. An expanded research programme, alongside the continuation of existing monitoring, would allow for a more holistic understanding of diversity, endemism, threats, and stresses within the Park. Longer-term datasets would allow for a greater understanding of how certain threats impact the ecology of cloud forest ecosystems (e.g. climate change), and therefore enable more effective monitoring strategies for various taxa. Completing IUCN threat assessments for as-yet-unassessed micro-endemics is also a priority.

## Conclusions

This review demonstrates CNP to be of exceptional ecological importance. However, despite its status as a protected area, it remains highly threatened by deforestation, poaching, disease, and climate change. There is some evidence that current conservation actions may mitigate some of these threats. However, these are not sufficient to secure the integrity of CNP's ecosystems, which continue to be degraded rapidly. Urgent short-term conservation actions relating to enforcement, deterrents, and species rescues are required, alongside a suite of interventions and research addressing and understanding underlying socio-economic dynamics in the Park in the longer term, to limit the impacts of deforestation and poaching. Amphibian disease and climate change are harder to manage at local levels and require multi-faceted responses from the international community, although *ex-situ* conservation actions and reforestation projects represent two partial means of mitigating these respective threats. Without immediate implementation of these conservation actions, it is highly likely that the unique cloud forest ecosystem of CNP will experience species extinctions and extirpations, and severe loss of ecosystem services, in the coming decades.

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# Bat assemblages and their ectoparasites in a Honduran cloud forest: Effects of disturbance and altitude

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## Abstract

The high diversity and endemism of cloud forests make them good models to explore the impacts of habitat disturbance on bat communities and their ectoparasites. Although bat responses to forest disturbance have been intensively studied, the response of their ectoparasites in cloud forests remains poorly known. We explore this knowledge gap by analyzing data gathered from 44 nights during a June-August field season in 2019 within Cusuco National Park, a protected cloud forest located in northwestern Honduras. Mist-netting was conducted at five recording stations between 1,300 and 1,925 m a.s.l., spanning a mix of closed-canopy forest and human-induced forest clearings. In total, 584 bats representing 36 species were identified. An overall ectoparasite prevalence of 41% was recorded from all bat captures. Prevalence was similar between males and females, although juveniles displayed a higher rate than adults, and some species were more prone to parasitism than others. Parasite prevalence was positively correlated with altitude. In lower montane forest, bat assemblages and parasite prevalence did not differ between closed-canopy forest and forest clearings. Conversely, in upper montane forest, bat diversity and parasite prevalence rates were higher in forest clearings. Overall, our results suggested that the effect of forest clearance varied with elevation and bat species. Forest clearance in high-altitude forests may have severe impacts on bat communities, not only reducing their diversity, but also increasing susceptibility to parasite infection, with its associated debilitating effects.

## Resumen extendido

**Antecedentes:** La alta diversidad y endemismos que presentan los bosques nublados los hacen modelos perfectos para explorar los impactos que tiene la deforestación en el hábitat de las comunidades de murciélagos y de sus ectoparásitos. Aunque las respuestas de los murciélagos a la deforestación han sido ampliamente estudiadas, se sabe muy poco acerca de la respuesta de sus ectoparásitos. A menudo, la respuesta de los murciélagos a actividades antropogénicas depende de la especie y la estructura del paisaje. Por ese motivo, nuestro objetivo fue evaluar el efecto de la tala de bosques en los ensamblajes de murciélagos y en la prevalencia de ectoparásitos en las áreas de bosques montanos bajos y altos en el Parque Nacional Cusuco, un bosque de niebla protegido en el noroeste de Honduras.

**Métodos:** Entre los meses de junio y agosto del 2019 se realizaron muestreos con redes de niebla en cinco estaciones de investigación, localizados entre 1,300 y 1,925 m s. n. m., abarcando una mezcla de bosque con dosel cerrado y bosque con claros ocasionados por la tala. Por la noche se colocaron cinco redes en las estaciones de muestreo, se tomó la muestra de tres estaciones de manera simultánea, en promedio. Se procesó, marcó y recolectó ectoparásitos a cada murciélago capturado. Por otra parte, se analizó y comparó la composición y estructura de los ensamblajes de murciélagos mediante un análisis multidimensional no métrico (NMDS) y diversidad efectiva por números Hill. Así como también se comparó la prevalencia de los ectoparásitos influenciados por la fragmentación, a nivel de la comunidad y entre las especies más comunes mediante comparaciones múltiples y regresión múltiple binomial.

**Resultados:** Un total de 584 murciélagos fueron identificados satisfactoriamente, pertenecientes a 36 especies y cinco familias, siendo la familia *Phyllostomidae* con mayor abundancia y riqueza. Se recolectaron ectoparásitos de 22 especies de murciélagos y se registró una prevalencia general de ectoparásitos del 46% de todas las capturas de murciélagos. La prevalencia fue similar entre machos y hembras, aunque los juveniles mostraron una tasa más alta que los adultos, y algunas especies fueron más propensas al parasitismo que otras. Además, se observó que la prevalencia del ectoparásito se correlacionó significativamente con la altitud. El ensamblaje de los murciélagos, en bosques de montaña baja no se encontraron diferencias significativas entre hábitats, en cuando a la diversidad y composición. Sin embargo, en los sitios de mayor altitud, los bosques con dosel cerrado presentaron una mayor diversidad en comparación con los sitios de bosque con claros ocasionados por la tala bosques. Este mismo patrón se observó en la prevalencia de ecotparásitos, pero las tasas de prevalencia fueron mayores en los claros ocasionados por la tala de bosque de montaña alta.

**Discusión:** El efecto de los sitios perturbados en los ensamblajes de los murciélagos varía entre elevaciones, provocando diferencias estructurales en las comunidades y en el dominio de las especies. Por otra parte, la prevalencia de ectoparásitos en individuos juveniles pudo deberse a la composición de refugios, hábitos sociales o de acicalamiento. La tala de árboles de los bosques montanos altos puede tener graves impactos en las comunidades de murciélagos, no solo reduciendo su diversidad, sino también aumentando la susceptibilidad a la infección por parásitos. Existe una creciente preocupación por la pérdida continua de biodiversidad en los bosques nublados. Una mejor comprensión de estas funciones de respuesta a la presión podría proporcionar herramientas para integrar el desafío de adoptar los sistemas socio-ecológicos en los programas de conservación en Mesoamérica.

# Introduction

A distinctive feature of the Mesoamerican biodiversity 'hotspot' (Myers, 2003; Myers *et al.,* 2000) is its cloud forest ecosystems, which occur from southern Mexico to Panama. Because of its geological history and varied topography, Central America and its cloud forests exhibit high biodiversity and endemism rates (CEPF, 2020; Powell & Palminteri, 2001). Cloud forests typically represent 'islands in the sky'; patches of habitat with cooler, moister conditions compared with lower altitude forests. This distinctive habitat, coupled with their biogeographical isolation, has led to the evolution of many species unique to these forests (Helmer *et al.*, 2019), including within its bat communities. Cloud forest bat communities as a whole provide important ecosystem services, acting as seed dispersers, pollinators, and predators of pest species (Fleming, 1993; Kalko *et al.*, 1996; Medellín *et al.*, 2000). The diversity of bats in terms of both species and functional roles also contributes to the diversity of parasites that live on them.

Due, in part, to their ecological adaptations (specialist feeding guilds, flight, communal roosting), bats are parasitized by many groups of arthropods (Simmons & Conway, 2003), such as mites, ticks, fleas, bugs, and bat flies (Dick & Graciolli, 2013; Lourenço & Palmeirim, 2008; Marshall, 1982, reviewed in Haelewaters *et al.*, 2018, 2020). Of these, bat flies are the best known and best studied. Bat flies are highly specialized ectoparasites that are exclusively associated with bats. They live in the fur and on wing membranes and feed on the host's blood. While bat flies are currently split into two families, Nycteribiidae and Streblidae, recent phylogenetic studies found that Streblidae is not a monophyletic group (Dittmar *et al.*, 2006). The nycteribiids, with about 276 described species in 11 genera, are most species-rich in the Eastern Hemisphere, whereas streblid bat flies (~240 species, 33 genera) are richer in the tropics and subtropics of the Western Hemisphere (Dick *et al.*, 2016; Graciolli & Dick, 2018; Haelewaters *et al.*, 2020).

The ecology of parasites remains poorly studied in most tropical regions and the effects of habitat loss and fragmentation on parasites are poorly understood. Studies that have explored this question have presented ambiguous patterns. Proximity to forest edges has been reported to lead to a reduction of ectoparasites in rats (Kiene *et al.,* 2020), which is consistent with the findings of Martínez-Mota *et al.* (2018), who studied parasite eggs in the stool of howler monkeys. On the other hand, Hiller *et al.* (2020), studying bat flies in Costa Rica, found that the direction of response to habitat types was host- as well as parasite-specific. Bat parasites such as bat flies can themselves be associated with numerous bacteria, viruses, blood parasites, and fungi (Liu *et al.*, 2020; Szentiványi *et al.,* 2019). Studies have shown their potential as vectors for disease agents such as Bartonella and Ledanteviruses (Dick & Dittmar, 2014; Goldberg *et al.,* 2017).

Mesoamerican cloud forests represent a good model for exploring the impacts of habitat disturbance on communities of bats and their parasites. Firstly, it is likely that many species found here are locally endemic, given high endemism in other taxa and the high levels of host-specificity displayed in many bat parasites. As such, these endemic species are likely to represent important conservation priorities for which novel data is particularly useful. Secondly, Mesoamerican cloud forests are heavily impacted by habitat destruction and degradation, experiencing some of the most severe rates of forest loss (relative to their overall area) of all Central American ecosystems (Cayuela *et al.*, 2006; Hansen *et al.*, 2020). Due to these pressures, it is necessary to conduct further research into their biodiversity, interactions, and responses to human disturbance.

Although bat responses to forest disturbance have been intensively studied, the influence of deforestation on bat ectoparasite prevalence in cloud forests remains poorly known. Moreover, these bat responses are often species-specific, as well as sensitive to spatial scale, degree of deforestation, and landscape matrix (Meyer *et al.*, 2016). At the population level, studies suggest that abundance responses to fragmentation are highly species- and guild-specific. Where abundances in frugivorous and nectivorous bat species often increase with disturbance, gleaning and carnivorous and insectivorous species typically decrease in abundance (Meyer & Kalko, 2008; Sampaio *et al.*, 2003). On the other hand, at the assemblage level, a positive or negative response depends on the structure, type of fragmentation, and habitat (Faria *et al*., 2006; Farneda *et al*., 2015). Bat assemblages vary along elevation gradients, and it is highly likely that they also vary along disturbance gradients in cloud forest ecosystems as well. However, the relative importance of these two variables for driving composition of bat communities and their ectoparasites remains poorly explored in Mesoamerican cloud forests. Therefore, our primary aim was to assess the effect of altitude and human-induced forest clearance on bat assemblages and their ectoparasites in lower and upper montane forests, as a first step to understanding the impact of these environmental gradients. While the ectoparasites have not yet been identified, we were also interested in parasite prevalence on different bat species, and how prevalence is affected by age of the bat host, reproductive status, altitude, and anthropogenic forest clearance. In this study, we present the results of our mist-netting surveys during field season 2019 at Cusuco National Park (CNP), a protected cloud forest in northwestern Honduras, where deforestation has rapidly accelerated in recent years, especially in the western side of the park (Martin *et al*., 2021).

# Methods

## Study area

Cusuco National Park (CNP, located at 15°32'31"N 88°15'49"W) is a 23,440-ha protected area of montane cloud forest located in the Sierra de Omoa, part of the Merendón mountain range in northwestern Honduras. The park spans an altitudinal range of 500–2,242 m a.s.l. and is divided into 7,690 ha of core zone and 15,750 ha of buffer zone, where some limited agriculture and other land use is permitted (Martin & Blackburn, 2009). The area receives an average of 2,788 mm of rain annually (Fundación Ecologista, 1994) and has a mean summer day time temperature (June–July) ranging from 21 °C at 1,150 m a.s.l. (range: 18–23.5 °C) to 15 °C at 2,200 m a.s.l. (13–16.5 °C) (Jones *et al*., 2020). The park represents a mosaic landscape with four categories of forest: tropical lowland dry forest, tropical moist forest, montane (cloud) forest, and 'bosque enano' or dwarf forest found above 2,000 m a.s.l. (Hoskins *et al*., 2018), although only small fragments of the first two categories remain in the buffer zone of the park.

## Mist-netting

As part of the long-term biodiversity monitoring efforts by Operation Wallacea (Gilroy *et al*., 2017), we conducted mist-netting surveys at five camps (recording stations) from June 8th to August 1st, 2019, located between 1,300 m and 1,925 m a.s.l. inside the core zone of CNP. Within each camp, depending on the local topography, we established three to five fixed-location mist-netting sites between 300 and 600 m apart (a total of 19 mist-netting sites). Three camps were sampled per night for six nights per week. For each survey session at each site, we used five nets (four 6 m × 2.6 m, one 12 m × 2.6 m nets, all with 36-mm mesh and five shelves) placed along trails and streams that acted as natural corridors. Mist nets were opened before sunset and checked every 15–30 minutes for six hours. Once a mist-netting site was sampled, it was not surveyed again for at least three days. We did not survey during nights with heavy rainfall. Sampling effort was calculated by summarizing the total meters of net and multiplying this by the number of hours nets deployed per night and presented as $m^2h$ (Straube & Bianconi, 2002).

We processed, marked, and released bats at the location of capture following previously described protocols (Sikes, 2016). Processing included species identification, determination of the sex, relative age (juvenile or adult), determined by the degree of ossification of the metacarpal-phalangeal joints (Baagøe, 1977), and reproductive status (female: non-reproductive, pregnant, lactating, post-lactating; male: scrotal, non-scrotal). We also measured the forearm length (mm) and body mass (g) and marked captured bats by clipping a small patch of dorsal fur. This temporary marking procedure is a useful and harmless technique for identifying recaptured individuals. We identified individuals to species-level using taxonomic keys (Medellín *et al*., 2007; Mora, 2016; Timm & Laval, 1998). Bat taxonomy follows

Tejedor (2005) (for *Natalus lanatus*), Velazco & Simmons (2011) (for *Sturnira parvidens*), Mantilla-Meluk & Muñoz-Garay (2014) (for *Myotis pilosatibialis*), and IUCN (IUCN, 2020) (for all other bat species).

## Ectoparasite collection

Collection of ectoparasites (bat flies and mites) was conducted following methods described by Walker *et al.* (2018). In order to collect fast-moving bat flies from their bat hosts, we first applied 96% ethanol with a paintbrush to slow them down. We removed ectoparasites using rigid Swiss Style Forceps #5 with superfine tip (BioQuip #4535, Rancho Dominguez, CA, USA) or Featherweight Forceps with narrow tip (BioQuip #4748). Some ectoparasites were collected by hand. Upon collection, ectoparasites were immediately transferred to 1.5 mL Eppendorf tubes or 0.2 mL PCR tubes (one tube per bat host) filled with 96% ethanol for preservation and long-term storage.

## Data analysis

We constructed species accumulation curves with 1000 randomizations for observed and estimated species richness (Jackknife 2 and Bootstrap) for CNP by considering each night as an independent sampling occasion. Then we evaluated the efficiency of the sampling effort and the representation of bat communities in the different camps by the estimation of Hill numbers. With this approach, by providing estimators for inter- and extrapolation, it is possible to compare different sample sizes. This extrapolates the smallest samples and compares species richness estimates at equal sampling completeness (Chao *et al.*, 2014). To compare the proportions of parasite prevalence between sexes and ages we applied Chi-squared tests.

To compare the bat community and ectoparasite prevalence between closed-canopy and anthropogenic forest clearing sites, we took three main approaches: 1) first, we performed a Nonmetric Multidimensional Scaling (NMDS) using Bray–Curtis dissimilarity; 2) then we estimated the diversity via Hill numbers; 3) finally, we compared the relative abundance and parasite prevalence at assemblage level and for the most commonly captured species. We determined the relative abundance for each species in each site using the following equation: RAspi = Total captures ÷ mnh × 100.

RAsp*i* = relative abundance for a species "i"; total captures = number of captures per species in a site; mnh = total number of hours that the nets remained open (Medellín *et al.*, 2000). The results were multiplied by 100 to correct for small values (de Carvalho *et al.*, 2019).

Because bat assemblages differ significantly with respect to elevation (Medina-van Berkum *et al.*, 2020), we conducted two separate analyses, one considering the camps in lower montane forest (1,200–1,400 m a.s.l., Guanales and El Cortecito) and another considering the camps in upper montane forest (1,500–1,700 m a.s.l., Base Camp and El Danto). We estimated Hill numbers using individual-based abundance rarefaction and extrapolation (Hsieh *et al.*, 2016). The following diversity measures were calculated: $q0$ (species richness), which is insensitive to species abundance; $q1$ (the exponential of Shannon entropy, referred here as Shannon diversity), which measures the number of common species in a community, (Chao *et al.*, 2014). Species diversity profiles were compared through overlapping confidence intervals (5–95%). To test for differences in species composition between closed-canopy forest and forest clearing sites, we used an Analysis of Similarity (ANOSIM), based on Bray-Curtis dissimilarity distances of species relative abundance. ANOSIM provides a $r$ value between 0 (minimum similarity) and 1 (maximum similarity). A $p$-value $< 0.05$ indicates that the results of the analysis are significant.

Finally, to test whether relative abundance and parasite prevalence was influenced by human-induced clearings at assemblage level and at species-specific level, Mann–Whitney U-tests were conducted. Additionally, a GLM with a binomial distribution was performed, where presence of ectoparasites was the binomial dependent variable (1 = parasites, 0 = no parasites) and disturbance degree, age, and sex were the independent variables. To correct for the unequal sampling effort per site, a variable log (effort) as offset was included in the formula (Hosmer *et al.*, 2013).

For all analysis we excluded all recaptured bats, and all statistical analyses were performed using R software version 3.6.1 (R Core Team, 2020) and the packages “vegan” (Oksanen *et al.*, 2019) and “iNext” (Hsieh *et al.*, 2016). To assess the Nonmetric Multidimensional Scaling, we used PAlaeontological STatistics (PAST) 2.17c software (Hammer *et al.*, 2001).

## Results

**Bat assemblages.** We captured a total of 590 bats (40,912 $m^2h$), of which 584 could be reliably identified. The remaining 1% escaped before they could be fully identified. The rate of recapture was 7.7% (42 bats) and varied among sites (Table 1). Our study sample of bats represented 36 species in 26 genera, belonging to five families. Species accumulation curves did not show a stabilizing trend, indicating that the number of species would have increased if we had sampled more nights (Figure 1). Phyllostomidae was the most abundant and speciose family, with 81% of the total captures representing 27 species; followed by Vespertilionidae (16%), Mormoopidae (3%), and Natalidae and Molossidae, which each represented < 1% of total captures. *Sturnira hondurensis* (n = 106) was the most commonly captured species, followed by *Artibeus jamaicensis* (102), *Myotis pilosatibialis* (74), *Dermanura tolteca* (70), and *Glossophaga soricina* (29), together compromising 72% of total captures. Ten species (28% of all species) were captured once during the entire 2019 field season (Table 1).

Overall, the proportion of captured males and females was similar. Adults accounted for 77% of the captures. For those species with more than five captures (n = 15), the adult sex ratio (male: female) ranged from 3:1 (*Bauerus dubiaquercus*) to 1:3 (*G. soricina*). Palpably pregnant individuals were recorded 88 times for 20 species (71% of the species recorded) and evidence of lactation was recorded in 35 females of 12 species (Table 1). The proportion of pregnant females captured varied among species. Among the commonly captured bats, 68% of adult females of *G. soricina* were pregnant in the surveyed period, 59% of *A. jamaicensis*, but only 33% of *S. hondurensis* and 4% of *M. pilosatibialis*.

**Bat ectoparasites.** Excluding bats that represented recaptures and bats that escaped or were released before processing for a variety of reasons, ectoparasites were recorded on 240 out of 521 screened bats (overall prevalence 46%), representing 22 bat host species (Table 1). Parasite prevalence by bat species ranged from 8% in both *Glossophaga commisarisi* and *Hylonycteris underwoodi* to 91% in *Pteronotus mesoamericanus*. In general, the parasite prevalence between female and male bats was similar (females 48%, males 43%). However, males of *G. soricina* and *D. tolteca* had a higher parasite prevalence compared to females (Chi-squared test, *G. soricina*: $x^2$ = 10.15, $p$ = 0.001; *D. tolteca*: $x^2$ = 3.18, $p$ = 0.007), whereas *A. jamaicensis* females had a higher prevalence than males (Chi-squared test, $x^2$ = 4.61, $p$ = 0.032). Taking into account the age of the bats, juveniles had higher parasite prevalence compared to adults (juveniles 57%, adults 42%; Chi-squared test, $x^2$ = 4,891, $p$ = 0.027). The relationship between parasite prevalence and reproductive status was marginally significant (Chi-squared test, $x^2$ = 5. 95, $p$ = 0.051), with reproductive adults having a decreased parasite prevalence (Bonferroni post hoc, $p$ = 0.015) compared to non-reproductive juvenile and adult individuals.

### Effect of human-induced forest clearings on bat assemblage and ectoparasite prevalence.

The comparison of bat assemblages between closed-canopy and human-induced forest clearing sites showed different patterns for lower versus upper montane forest sites. For sites in lower montane forest, the bat assemblages did not differ significantly between habitat types ($R^2_{ANOSIM}$ = 0.18, $p$ = 0.153). The lower r value suggests that dissimilarities in mist-netting sites within recording stations were greater than between habitats. On the other hand, for upper montane forest sites, bat assemblages differed between closed-canopy and human-induced forest clearings ($R^2_{ANOSIM}$ = 0.8, $p$ = 0.002; Figure 3a–b).

In terms of species diversity, the effect of human-induced forest clearings also showed different patterns between lower and upper montane forest

(Figure 3). The comparison of species richness (*q*0) showed that lower montane forest sites did not differ significantly between disturbance types. However, in upper montane forest, closed-canopy forest sites had significantly higher richness compared to sites with human-induced forest clearings (Figure 3c–d). Taking into account the relative abundance values of all species (Shannon diversity (*q*1)), species diversity showed similar patterns. However, once the dominant species was accounted for (Simpson diversity (*q*2)), species diversity indices showed different patterns (Figure 3e–f). Bat assemblages were dominated by a smaller number of species in closed-canopy forest sites. Conversely, in upper montane forest sites, closed-canopy sites harbored a higher diversity of bats compared to forest clearings. Of the five most commonly captured bats at lower montane forest sites, *S. hondurensis* was significantly more abundant in human-induced clearings, whereas *A. jamaicensis* and *D. tolteca* were more abundant in closed-canopy forest. On the other hand, in upper montane sites, *D. tolteca* was only present in closed-canopy forest, whereas *C. sowelli* was more abundant in human-induced forest clearings habitats (Figure 2).

In general, upper montane sites had higher parasite prevalence compared to lower montane sites (Mann-Whitney U Test, U = 58, p = 0.016, Figure 4). Parasite prevalence did not differ between closed-canopy and human-induced forest clearings habitats in lower montane forest sites (Mann-Whitney U Test, U = 6, $p = 0.685$); but it did at upper montane forest sites, where human-induced forest clearings habitats had higher prevalence rates compared to those in closed canopy forest (Mann-Whitney U Test, U = 17, $p = 0.047$), although this varied among bat species (Table 2). For example, prevalence of ectoparasites on *M. pilosatibialis* was higher in forest clearings (GLM, $p = 0.018$). On the other hand, parasite prevalence for *S. hondurensis* (GLM, $p = 0.265$) did not differ among habitats but did differ between ages (GLM, $p = 0.024$), where juveniles had higher parasitism rate compared to adults. In lower montane sites, parasite prevalence *in S. hondurensis* was higher in closed-canopy forest habitats (GLM, $p = 0.038$), but no such difference was observed for other common species such as *A. jamaicensis* or *G. soricina* (GLM, $p = 0.72$ and $p = 0.06$, respectively).

# Discussion

Cusuco National Park has been listed as the 123rd most irreplaceable protected area in the world (Le Saout *et al.,* 2013). However, in recent years illegal logging within the park has intensified, impacting species populations and overall community composition (Hoskins *et al.,* 2018; Martin *et al.,* 2021). In a period of 44 survey nights, we recorded 61% of the known bat species in CNP (Medina-van Berkum *et al.,* 2020) and our results showed that bat assemblages were affected by anthropogenic forest clearance, particularly in upper montane forest sites. This pattern was also correlated with ectoparasite prevalence; sites within anthropogenic forest clearings had higher ectoparasite prevalence than closed-canopy forest sites, however the effect was also largely species-specific.

Overall, parasite prevalence did not differ between sexes and among reproductive statuses. Prevalence did, however, vary among bat species, suggesting species-specific preferences of ectoparasites (Hiller *et al*., 2020; Pilosof *et al*., 2012). We found variation in ectoparasite prevalence between sexes of three bat species: *A. jamaicensis*, *D. tolteca*, and *G. soricina*. High prevalence in female hosts (*G. soricina* and *D. tolteca*) might be due to the longer time that they tend to spend in their roost to provide maternal care (Esbérard *et al*., 2012; Patterson *et al*., 2008a), whereas high prevalence in males might be an ectoparasite dispersion strategy, as male bats will disperse to form new colonies (Bertola *et al*., 2005). Prevalence may also be influenced by social behavior patterns and roosting habits of individual bat species. Patterson *et al*. (2007) found that bats roosting in more permanent structures had increased measures of parasitism (parasite prevalence, number of bat fly species per bat).

Moreover, our results showed that juvenile individuals had higher prevalence of ectoparasites compared to adults. This is consistent with the results of a preliminary study on bat ectoparasites in CNP (Stars, 2017), which found that 78% of the captured juveniles had ectoparasites compared to only 50% of adults. Higher parasite prevalence in juveniles is

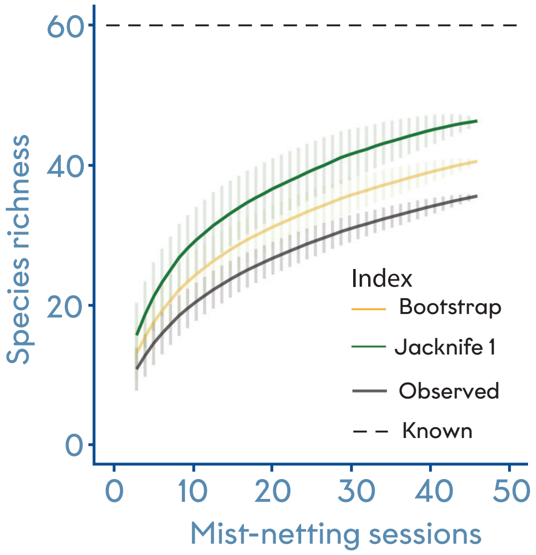

**Figure 1.** Species accumulation curve of bats captured in Cusuco National Park, north-western Honduras, between June and August 2019. The black curve indicates observed richness. Non- parametric species richness estimator values are shown in the green curve (Jackknife1) and yellow curve (Bootstrap) with 95% of confidence intervals. The black broken line represents the total number of bats species known for the park based on 15 years of survey effort (Medina-van Berkum *et al.*, 2020).

TABLE 1. List of bats species captured in Cusuco National Park, north-west Honduras

| Species | Recording stations at Cusuco National Park | | | | | | |
|---|---|---|---|---|---|---|---|
| | GU | CO | BC | DA | CA | Total | EC |
| Phyllostomidae | | | | | | | |
| *Anoura geoffroyi (N)*[+] | 0 | 0 | 4 | 0 | 0 | 4 | Yes |
| *Artibeus jamaicensis (F)** | 88 | 14 | 5 | 1 | 0 | 108 | Yes |
| *Carollia sowelli (F)*[+] | 7 | 7 | 6 | 5 | 0 | 25 | Yes |
| *Carollia subrufa (F)* | 1 | 0 | 1 | 0 | 0 | 2 | Yes |
| *Centurio senex (F)*[+] | 1 | 0 | 0 | 0 | 0 | 1 | No |
| *Chiroderma salvini (F)** | 2 | 0 | 0 | 0 | 0 | 2 | Yes |
| *Choeroniscus godmani (F)* | 0 | 0 | 1 | 0 | 0 | 1 | No |
| *Dermanura azteca (F)* | 3 | 0 | 2 | 0 | 0 | 5 | Yes |
| *Dermanura phaeotis (F)*[+] | 2 | 0 | 2 | 0 | 0 | 4 | No |
| *Dermanura tolteca (F)**[+] | 31 | 5 | 29 | 0 | 1 | 72 | Yes |
| *Dermanura watsoni (F)**[+] | 3 | 1 | 3 | 0 | 1 | 8 | Yes |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Desmodus rotundus (S)* | 2 | 0 | 5 | 0 | 0 | 7 | Yes |
| *Diphylla ecaudata (S)* | 0 | 0 | 1 | 0 | 1 | 2 | No |
| *Enchisthenes hartii (F)**+ | 3 | 3 | 1 | 0 | 0 | 7 | Yes |
| *Glossophaga commissarisi (N)** | 6 | 2 | 3 | 2 | 0 | 13 | Yes |
| *Glossophaga leachii (N)** | 3 | 0 | 1 | 0 | 0 | 4 | Yes |
| *Glossophaga soricina (N)** | 14 | 12 | 3 | 0 | 0 | 29 | Yes |
| *Hylonycteris underwoodi (N)**+ | 0 | 6 | 6 | 0 | 0 | 12 | Yes |
| *Micronycteris minuta (I)* | 0 | 1 | 0 | 0 | 0 | 1 | No |
| *Micronycteris schmidtorum (I)* | 0 | 0 | 1 | 0 | 0 | 1 | No |
| *Phyllostomus hastatus (O)**+ | 0 | 0 | 2 | 0 | 0 | 2 | No |
| *Platyrrhinus helleri (F)** | 1 | 0 | 0 | 0 | 0 | 1 | No |
| *Sturnira hondurensis (F)**+ | 4 | 18 | 58 | 19 | 8 | 107 | Yes |
| *Sturnira parvidens (F)** | 0 | 0 | 0 | 0 | 2 | 2 | No |
| *Trachops cirrhosus (C)** | 0 | 1 | 0 | 1 | 0 | 2 | Yes |
| *Vampyressa thyone (F)* | 1 | 0 | 0 | 0 | 0 | 1 | No |
| *Vampyrodes major (F)** | 7 | 0 | 5 | 0 | 0 | 12 | Yes |
| Vespertilionidae | | | | | | | |
| *Bauerus dubiaquercus (I)** | 3 | 0 | 5 | 0 | 0 | 8 | Yes |
| *Eptesicus brasiliensis (I)** | 0 | 2 | 3 | 0 | 0 | 5 | Yes |
| *Eptesicus furinalis (I)* | 0 | 0 | 1 | 0 | 0 | 1 | No |
| *Lasiurus frantzii (I)* | 0 | 0 | 1 | 0 | 0 | 1 | No |
| *Myotis pilosatibialis (I)**+ | 1 | 1 | 52 | 12 | 9 | 75 | Yes |
| Molossidae | | | | | | | |
| *Molossus rufus (I)** | 1 | 0 | 0 | 0 | 0 | 1 | No |

|  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
| Mormoopidae |  |  |  |  |  |  |  |
| *Pteronotus davyi (I)*+ | 2 | 0 | 3 | 0 | 0 | 5 | No |
| *Pteronotus mesoamericanus (I)** | 0 | 1 | 6 | 5 | 0 | 12 | Yes |
| Natalidae |  |  |  |  |  |  |  |
| *Natalus lanatus* (I) | 0 | 0 | 1 | 0 | 0 | 1 | Yes |
|  |  |  |  |  |  |  |  |
| Captures | 191 | 74 | 210 | 45 | 22 | 542 |  |
| Species | 21 | 14 | 28 | 7 | 6 | 36 |  |
| Elevation m a.s.l. | 1287 | 1400 | 1572 | 1591 | 1825 |  |  |
| Unique species | 5 | 1 | 0 | 8 | 1 |  |  |
| Effort m$^2$h | 8,872 | 5,692 | 16,912 | 4,185 | 5,25 | 40,912 |  |
| Capture rate | 0.022 | 0.013 | 0.012 | 0.010 | 0.004 | 0.013 |  |
| Recapture rate | 13 (6.8%) | 3 (4.1%) | 21 (10%) | 2 (4.4%) | 3 (13.6%) | 42 (7.7%) |  |
| Individuals with ectoparasites | 80 | 27 | 96 | 27 | 10 | 240 |  |

Table 1. List of bats species captured in Cusuco National Park, Honduras in June-July 2019, with information on captures per bat species (recaptures removed), camp elevation, survey effort, and recapture and parasitism rates per camp (GU = Guanales, CO = El Cortecito, BC = Base Camp, DA = El Danto, CA = Cantiles). EC indicates species from which ectoparasites were collected. Letters in parenthesis after each name indicates primary dietary guild as defined by Kalko *et al.* (1996) and Soriano (2000): C = carnivorous, F= frugivorous, I = insectivorous, N= nectivorous, S= sanguivorous. Underlined names represent species listed as Threatened (Hernández 2015, IUCN 2020). An asterisk (*) indicates that pregnant females were captured and plus (+) indicates that lactating females were captured.

also reported in Mexican, Panamanian, and Venezuelan sites (Hiller *et al.*, 2020; Patterson *et al.*, 2008b; Tlapaya-Romero *et al.*, 2015). This may be linked to several factors, such as stable roosts and a close relationship between mother and offspring allowing for ample opportunities for parasite transmission events. In addition, young individuals have thinner skin, their immune system is still underdeveloped, and they are deficient in their grooming skills (Bertola *et al.*, 2005; Dick & Patterson, 2007; Hiller *et al.*, 2020).

The effect of small human-induced forest clearings on bat assemblages varied between elevations and this effect was species-specific. Whereas some studies have shown that deforestation has little or no effect on species richness (Meyer & Kalko, 2008; Ochoa, 2000), others have reported structural differences in the community and shifts in species dominance and ranks distribution (Clarke *et al.*, 2005; Peters *et al.*, 2006). Our results showed that in lower montane forest, species richness and Shannon diversity did not differ between habitats. However, Simpson diversity was significantly lower in closed-canopy forest, indicating that the bat assemblage was composed by one or a few dominant species. On the other hand, the effects of forest clearance were significantly stronger in upper montane forest, where human-induced forest clearings harbored few bat species and the assemblage was dominated by one or a few species, compared to closed-canopy forest. This negative effect on bat diversity at higher elevations might be related to food availability constraints (Castaño *et al.*, 2018; Soriano, 2000).

In lower montane forest, our results showed an opposite trend compared to previous studies, which found that fragmented areas tend to harbor few species of bat with assemblages being characterized by a small number of dominant species (e.g.: Meyer & Kalko, 2008; Sampaio *et al.*, 2003). The opposite pattern found in CNP might be related to the large number of *A. jamaicensis* captures in closed-canopy sites. Despite being known as a species tolerant of human disturbance, in this study, *A. jamaicensis* was less abundant in forest clearings than in closed-canopy sites. This may be due to the fact that the forest clearings are still at an early successional stage, where transformation of the land has not yet provided enough fruiting resources. In comparison, a higher number of captures of *A. jamaicensis* has been reported in Buenos Aires, a village located at the buffer zone of the park (Estrada-Villegas *et al.*, 2007; Thompson & Vulinec, 2017), particularly in shaded coffee plantations.

In addition to varied impacts on bat species resulting from human-induced forest clearing, the effect on ectoparasites was similarly variable. While, in general, upper montane forest sites had higher parasite prevalence rates compared to low montane forest sites, there was considerable variability in parasitism among bat host species and disturbance category within these altitude zones. In lower montane forests, overall parasite prevalence did not vary between habitat types, although individual species showed differing prevalence rates. While other common species showed no difference in parasite prevalence, *S. hondurensis* showed a high prevalence in closed-canopy forest habitat. Conversely, in upper montane forest, higher overall prevalence was recorded in human-induced forest clearings, especially for *M. pilosatibialis*. Bat behavior, availability of resources, and micro-climatic conditions of bat roosts are the fundamental factors that influence the changes in parasite prevalence and intensity (Hiller *et al.*, 2020). Habitat alterations, such as forest clearance, affect both roosting availability and resources and may have a positive effect on parasite prevalence by increasing roost crowding. The highly variable levels of parasite prevalence among habitat types, species, and altitudes at CNP reflects the findings of Brearley *et al.* (2013), who found that the relationships between wildlife diseases and human-modified landscapes are rarely consistent, and particularly so in rural agricultural landscapes.

Deforestation in CNP has accelerated rapidly in recent years (Martin *et al.,* 2021), which puts its bat communities in danger (Hernández, 2015). This is especially so at higher elevations, where species are more limited in their food resources (Castaño *et al.,* 2018). While the presence of a certain species does not necessarily imply population stability, it does suggest some tolerance of anthropogenic forest clearance. However, this is strongly influenced by the landscape matrix (Silva *et al.*, 2020). All forest clearing sites surveyed in this study were surrounded by closed-canopy forest, creating an edge effect, where bats could still use the resources of this forest. However, declines in species richness in forest

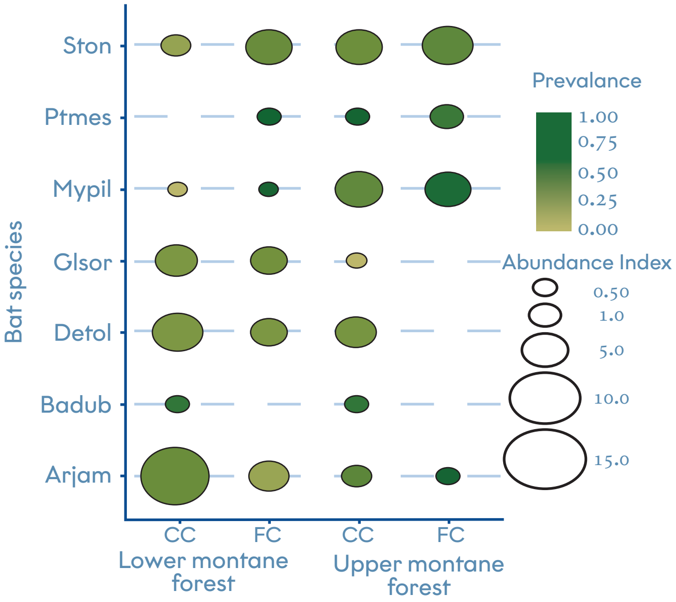

**Figure 2** Relative abundance and proportion of parasitism prevalence in the eight most abundant bat species captured in Cusuco National Park during the summer of 2019. CC indicates closed canopy sites, and FC indicates human-induced forest clearings. Empty spaces indicate that the species was not captured in the specific camp during the surveyed period. Bat species: Arjam: *Artibeus jamaicensis,* Badub: *Bauerus dubiaquercus,* Casow: *Carollia* sowelli, Detol: *Dermanura tolteca,* Glsor: *Glossophaga soricina,* Mypil: *Myotis pilosatibialis,* Ptmes: *Pteronotus mesoamericanus* and Sthon: *Sturnira hondurensis.*

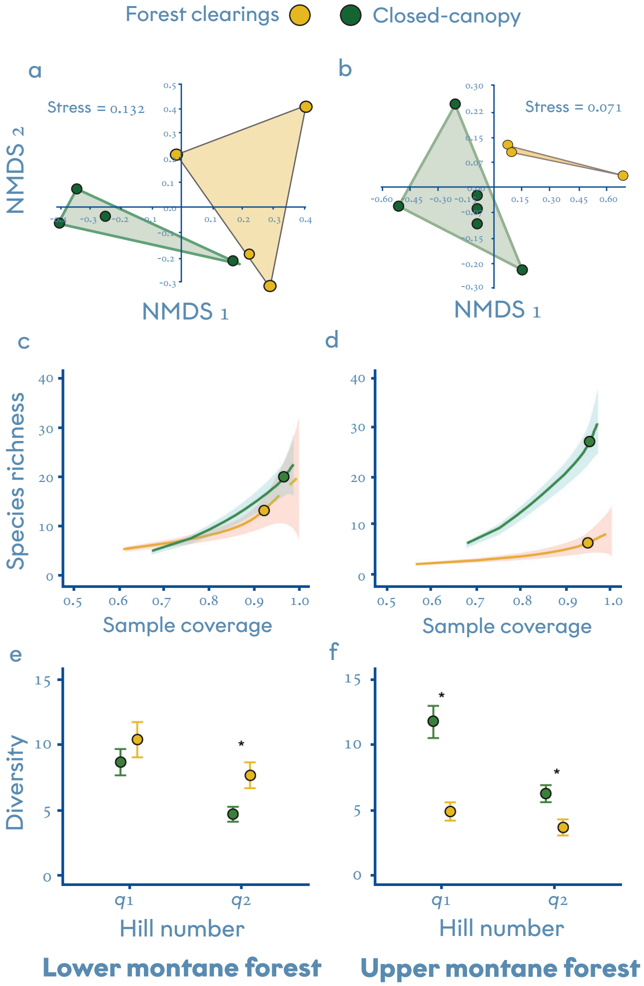

**Figure 3** Results of Nonmetric Multidimensional Scaling analyses using Bray–Curtis similarity, sample coverage and Hill numbers diversity (q1 and q2) comparing bat assemblages within Cusuco National Park, north-western Honduras. Bat assemblages at lower montane (left) and upper montane forest (right) were compared in closed-canopy (green symbols) and human-induced forest clearings (yellow symbols) sites. Stress numbers indicate the match between inter object distance (sites) and dissimilarity. The lower the stress value, the better the match. All extrapolation curves of the Hill numbers were plotted to a doubling in sample size, and 500 bootstrap replicates were used to estimate 95% confidence intervals.

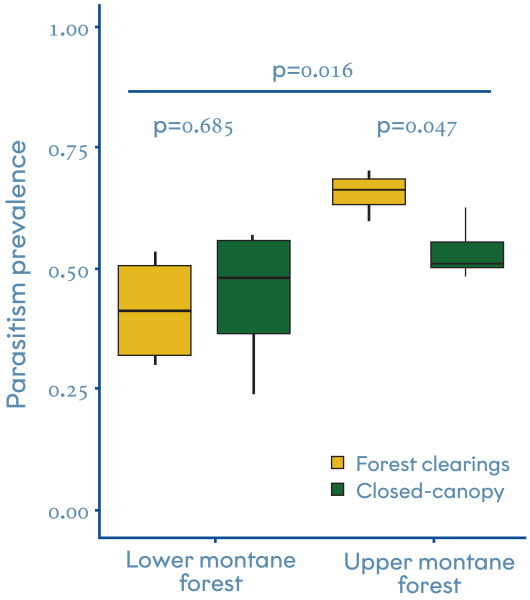

**Figure 4** Parasitism proportion in closed canopy and human-induced forest clearing habitats in Cusuco National Park, north-western Honduras. Proportion (Median, 1st and 3rd quartiles, SD) of bats with ectoparasites in lower and upper montane forest. Differences between habitat and vegetation type were tested with Mann-Whitney U test. P values < 0.05 indicate significant differences.

TABLE 2. Summary of generalized linear models with binomial logistic regression of parasitism prevalence within the five most common bat species captured in Cusuco National Park, Honduras

| LMF | *Artibeus jamaicensis* | | | *Sturnira hondurensis* | | | *Glossophaga soricina* | | |
|---|---|---|---|---|---|---|---|---|---|
| Predictors | OR | Z | *p* | OR | Z | *p* | OR | Z | *p* |
| Habitat | 1.30 | 0.36 | 0.720 | 0.05 | -2.07 | **0.038≠** | 0.17 | -1.83 | 0.067 |
| Sex | 0.33 | -2.02 | **0.043♀** | 0.73 | -0.31 | 0.755 | 2.05 | 0.67 | 0.504 |
| Age | 2.03 | 1.05 | 0.293 | 3.44 | 0.96 | 0.337 | 5.35 | 1.42 | 0.155 |
| UMF | *Myotis pilosatibialis* | | | *Sturnira hondurensis* | | | *Carollia sowelli* | | |
| Predictors | OR | Z | *p* | OR | Z | *p* | OR | Z | *p* |
| Habitat | 0.08 | -2.36 | **0.018≠** | 0.53 | -1.12 | 0.265 | 1.13 | 0.07 | 0.941 |
| Sex | 0.62 | -0.92 | 0.357 | 0.51 | -1.40 | 0.163 | N/A[a] | | |
| Age | 0.62 | -0.61 | 0.543 | 3.35 | 2.25 | **0.024¥** | 0.84 | -0.10 | 0.921 |

[a] Only male C. sowelli individuals were captured.

Table 2. Summary of generalized linear models with binomial logistic regression of parasitism prevalence within the five most common bat species captured in Cusuco National Park, Honduras. Effect of degree of deforestation, sex, and age on parasite prevalence in lower montane forest (LMF) and at upper montane forest (UPM). Odd ratios (OR), z and *p* values per species and predictors are shown. Symbols indicate a higher prevalence in human-induced forest clearing (≠), females (♀), and juveniles (¥).

edge habitats have been linked to habitat avoidance by animalivorous bats, because of flight constraints or increase of predation risk (Meyer & Kalko, 2008; Rocha *et al.*, 2017). Moreover, the effect of forest clearings largely depends on their size and successional stage. In this study, the studied forest clearings were less than five years old, and the response of bat assemblages and their relationship with ectoparasites to deforestation might change over time, depending on the land use or the regeneration rate of these habitats. Longer-term studies of logging impacts are therefore necessary to determine these impacts more thoroughly. Although the analyses were corrected by sampling effort, it is worth mentioning that the biases of sampling effort might still have affected the results of bat diversity estimates, increasing the potential of a type III error. Increasing survey effort not only increases the capture rate, but also the possibility of capturing rare species. Moreover, sites with human-induced clearings might reduce the efficiency of mist-nettings, since mist nets are more effective in locations with dense vegetation-containing corridors (Duffy *et al.*, 2000; Hourigan *et al.*, 2008).

In summary, the results of this study suggest that the effect of forest clearance varies with elevation and bat species. Forest clearings in high-altitude forests may have severe impacts on bat communities, not only reducing their diversity, but also increasing susceptibility to parasite infection. In order to gain a better understanding of the complex underlying mechanisms involved in the responses of bat assemblages and their ectoparasites to deforestation, long-term studies are required. Further research into this system should use complementary sampling methods and include environmental and disturbance variables across spatial and temporal changes (Rocha *et al.*, 2018), as well as include effects of food resources and physiological constraints on bat communities and their parasites (Meyer *et al.*, 2016). Moreover, further research needs to employ quantitative and multi-parameter approaches to evaluate the effect of human-induced forest clearings on taxonomic diversity, functional guild, behavior, immune systems, interactions with other organisms, and ecosystem services. There is an increasing concern about the ongoing loss of biodiversity in cloud forests and a better understanding of these pressure-response functions might provide tools to integrate the challenge of embracing socio-ecological systems in conservation programs in Mesoamerica.

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# *Ecnomiohyla salvaje* Hylidae (Wilson, McCranie & Williams 1985) in Cusuco National Park, Honduras: morphological descriptions of adults and metamorphs with notes on ecology, natural history, and the conservation implications of amphibian chytrid fungus (Bd)

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## Abstract

Cusuco National Park (CNP), a montane cloud forest in north-western Honduras, harbours what is thought to be among the last known populations of the Endangered Copán Fringe-limbed Tree Frog, *Ecnomiohyla salvaje*. This paper provides novel information that may inform the conservation status of this species whilst simultaneously building on its poorly known natural history. We add to the four total confirmed adult records from 1982-2014 by reporting an additional three adult individuals encountered between 2014 and 2018. Additionally, we provide the first descriptions of two late stage tadpoles (herein referred to as metamorphs), which adds to the information given on adults and earlier life stages in the species authority paper. We also correct and include formal photographs for the original known, but as yet 'unvouchered' *E. salvaje* observation in 2009 at CNP. We present morphometrics for most individuals encountered, as well as details of their natural habitat, defensive behaviour and ecology. Our observations of *E. salvaje* support the hypothesis that this canopy adapted species may be dependent on tree holes within intact forest. Being a tree-hole breeding species, the simultaneous presence of adult *E. salvaje* in tree holes with late stage tadpoles nearby, suggests that there may be an element of parental care towards offspring. Skin swabs from four *E. salvaje* individuals were tested via qPCR analysis for the presence of amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*); all these individuals tested positive for *Bd,* with infection loads ranging 0.24 - 53.96 Bd Genomic Equivalents. These results may have significant implications for the conservation of *E. salvaje*, considering chytridiomycosis likely led to severe population declines and/or the possible extinction of its congener *E. rabborum.*

## Resumen extendido

**Antecedentes y justificación:** *Ecnomiohyla salvaje* (Hylidae) es una especie de rana arborícola conocida solo en unas pocas localidades del sur de Guatemala y noroeste de Honduras. Habita específicamente la región de la Sierra del Merendón. Dentro de su distribución se encuentra el Parque Nacional Cusuco (PNC), un sitio reconocido como un área de especial importancia para la conservación de anfibios debido a su alto porcentaje de endemismo. El estilo de vida arborícola de esta especie dificulta estudios acerca de su ecología y población. Según la Unión Internacional para la Conservación de la Naturaleza (UICN), *E. salvaje* se categoriza como una especie en peligro de extinción debido a su limitada distribución dentro de un área que se encuentra amenazada por deforestación y pérdida de hábitat, el bajo número de observaciones y estudios, y la presencia de enfermedades que afectan anfibios en el PNC tal como la quitridiomicosis. Esta enfermedad es causada por el hongo patógeno *Batrachochytrium dendrobatidis* (*Bd*). Se especula que parte de la posible extinción de su congénere, *Ecnomiohyla rabborum,* fue resultado de la propagación regional de *Bd,* en conjunto con la deforestación y perturbación antrópica en el área que habitaba. Estudios previos realizados en el PNC han comprobado la presencia de hongo quítrido en otras especies de anuros, pero hasta la fecha no había sido posible realizar exámenes para detectar la presencia de *Bd* en *E. salvaje.* Debido a los pocos estudios sobre esta especie, cualquier información que permita un mayor entendimiento sobre su estado de conservación y ecología es de vital importancia. El presente estudio provee descripciones de la historia natural y morfología de los dos adultos y los dos renacuajos en últimas etapas de desarrollo que fueron observados entre el 2017 y 2018, al igual que las implicaciones que tendría la presencia de hongo quítrido en la conservación de *E. salvaje.* También informamos brevemente sobre un registro adicional de un adulto de *E. salvaje* en 2014, y agregamos detalles y fotografías adicionales para el registro de adulto confirmado anterior de 2009.

**Materiales y métodos:** Los individuos descritos en este estudio fueron encontrados en el PNC entre junio y agosto del 2017 y 2018 con la colaboración de Operation Wallacea. Las observaciones de *E. salvaje* en el PNC ocurrieron cerca de quebradas en dos campamentos de similar altitud (1287-1400 m s. n. m.) y tipo de bosque (bosque latifoliado montano bajo). Luego de la captura de los individuos de *E. salvaje*, se procedió a apuntar la fecha, hora, descripción de hábitat y del comportamiento observado. Asimismo, se tomaron distintas medidas morfométricas, y en el caso de adultos, se determinó el sexo. Posteriormente a la toma de datos y muestras de *Bd*, se fotografió y liberó a los individuos en el mismo sitio de captura.

Todos los individuos fueron procesados bajo técnicas asépticas. Se utilizaron hisopos estériles y guantes de nitrilo para tomar la muestra y determinar la presencia de *Bd* en los anfibios. A cada individuo se le realizó un frotis pasando el hisopo cinco veces en la parte ventral de las extremidades y parche bebedor. La parte superior de los hisopos se colocó dentro de viales de 2 ml conteniendo 500 µl de etanol (99%), y se mantuvieron refrigerados a -20 °C hasta que se realizó la extracción de ADN genómico. Se cuantificó el estado de infección y la carga de *Bd* mediante la técnica de reacción en cadena de la polimerasa en tiempo real (qPCR) para amplificar la región ITS1/5.8S del gen ribosomal. Como control positivo se utilizaron estándares de ADN de *Bd* (100, 10, 1, 0.1 de equivalentes genómicos de zoosporas), y agua destilada como control negativo. Todas las muestras fueron corridas en duplicado y luego se cuantificó la carga de *Bd* al comparar los valores de las muestras de qPCR con los valores obtenidos de los estándares de ADN. Se consideró como resultado positivo a todas las muestras en las que amplificaron ambos duplicados con una carga media superior a 0.1 equivalentes genómicos (GE).

## Resultados

Se observó un total de cuatro individuos de *E. salvaje* en las expediciones realizadas en el PNC durante 2017 y 2018. El primer individuo, observado en 2017, se hallaba en las últimas etapas de desarrollo (juvenil), lo cual se determinó mediante la presencia de una cola en proceso de absorción. Dos noches después se observó una hembra adulta sobre una rama a 5m de altura, posicionada 2m bajo una cavidad en el tronco del árbol. Al atrapar y procesar el individuo, se observó un comportamiento defensivo cuando la *E. salvaje* procedió a inflar su cuerpo. Asimismo, se presenció metacrosis 24 horas después de la captura. En 2018 se encontró un macho adulto dentro de un agujero en un árbol, cercano a donde se encontraba la hembra avistada en 2017, el cual también infló su cuerpo luego de ser procesado. A pesar de que el macho era de mayor tamaño, la hembra obtuvo un peso más alto. También se observó metacrosis en el cuarto individuo, el cual se encontraba en estado juvenil. Además, informamos brevemente de un registro adicional de un adulto de *E. salvaje* encontrado en 2014, y agregamos detalles y fotografías adicionales para el registro de un adulto hallado en 2009, el cual ya había sido confirmado anteriormente. Todos los individuos resultaron positivos para la presencia de *Bd*, mostrando cargas de infección entre 0.24-53. GE. Ambos adultos presentaron una carga de infección menor que los juveniles, el juvenil hallado en 2017 tuvo la mayor carga, era 225 veces más alta que la encontrada en la hembra observada ese mismo año.

**Discusión e implicaciones:** Algunos de los comportamientos observados —tal como los adultos que inflaron su cuerpo luego de ser manejados— han sido reportados para otras ranas arborícolas, pero no habían sido observados en esta especie. Este tipo de comportamientos en ranas de gran tamaño, tal como *E. salvaje*, son especialmente efectivos en contra de depredadores. Un mecanismo de defensa relacionado a este comportamiento es fragmosis, un proceso en donde los organismos inflan su cuerpo dentro de una cavidad y utilizan su cabeza para tapar la entrada y protegerse contra depredadores. A pesar de no haber sido visto en esta especie, se ha reportado este comportamiento en diversas ranas de la familia Hylidae que poseen cráneos co-osificados, tal como el que posee *E. salvaje*. Por ende, sugerimos que esta defensa podría ser utilizada por *E. salvaje,* aunque no podemos confirmarlo. El hallazgo del macho y la hembra cerca de cavidades en árboles soporta la idea de que utilizan estas áreas como refugio y sitio de reproducción, incluso como zonas para que los renacuajos puedan realizar metamorfosis ya que las cavidades pueden almacenar agua. Los dos juveniles fueron encontrados cerca de la cavidad del árbol donde se observaron los adultos, sugiriendo un posible cuidado parental. Además, esto insinúa que es posible que los adultos presenten fidelidad en cuanto a la elección de sitios de reproducción debido a la dificultad para encontrar árboles maduros con cavidades que posean las condiciones adecuadas.

Estudios anteriores sobre la presencia de *Bd* en ranas arborícolas sugieren que estas pueden ser potencialmente vectores de *Bd,* y que los refugios arbóreos que habitan pueden ser reservorios para este patógeno. La presencia de *Bd* en los cuatro individuos es alarmante, aunque cabe recalcar que el número de individuos muestreados fue demasiado bajo para poder hacer conclusiones sobre el estado de la población entera. Lastimosamente, además de la amenaza de esta enfermedad, los anfibios del PNC se encuentran bajo otras amenazas tal como la creciente deforestación y perturbación de la zona núcleo del parque. Considerando que es probable que la posible extinción de *E. rabborum* fue causada por *Bd*, sugerimos establecer un programa de reproducción en cautiverio para poder estudiar, proteger y luego reintroducir individuos de *E. salvaje.*

# Introduction

The genus *Ecnomiohyla* occurs in southern Mexico, throughout Central America, and into northern South America. It is comprised of twelve described species, eleven of which have been described since 1943, including four in the last 15 years (Campbell *et al.* 2000; Faivovich *et al.* 2005; Mendelson *et al.* 2008; Savage & Kubicki, 2010; Batista *et al.* 2014; Kubicki & Salazar, 2015). Frogs of this genus are well-adapted for an arboreal lifestyle and are characterised by having extensive foot webbing thought to help facilitate gliding and controlled falling in the forest canopy (Mendelson *et al.* 2008). In addition, they possess cryptic, textural camouflage and fleshy scalloped dermal fringing on their appendages, which affords them effective concealment when in contact with mossy tree branches and trunks. Relative to other genera, little is known about this genus due to its elusive canopy lifestyle, and some species have not been observed for many decades (Mendelson *et al.* 2015). While apparently rare, the perceived low abundance of these species is probably biased by the fact most regional amphibian surveys use traditional river search methods (Batista *et al.* 2014), as well as the overall lack of studies focusing on canopy ecology (Nakamura *et al.* 2017).

*Ecnomiohyla salvaje* (Wilson, McCranie & Williams 1985) is an elusive canopy specialist frog, known only to occur at two or three localities in north-western Honduras and south-eastern Guatemala from elevations of 1250 - 1450 m a.s.l. (IUCN SSC Amphibian Specialist Group 2020). In Honduras, owing to the degradation of forest habitat across its range, *E. salvaje* may be restricted to a single locality in the Sierra de Omoa (also called Sierra de Merendón) mountain range, Cusuco National Park (CNP). Situated near the western border between Honduras and Guatemala (Solis, et el., 2017; Wilson and McCranie 2001), the park encompasses altitudinal gradients up to 2425 m a.s.l. and a variety of habitats, predominantly consisting of cloud forest, dwarf forest (bosque enano) and rainforest composed of broad-leaf and pine (See: McCranie and Wilson, 2004; Townsend and Wilson, 2008; Gilroy *et al.* 2017; Martin *et al.* 2021). CNP was ranked in the top 25 most irreplaceable protected areas for threatened amphibian conservation worldwide (Le Saout *et al.* 2013), based on its high percentage of endemism and the fact that it harbours the only or largest remaining populations of many amphibian species, including *E. salvaje* (Solis *et al.* 2017). Furthermore, CNP falls within the "Sierra de Omoa - Cusuco" Alliance for Zero Extinction site, which are Key Biodiversity Areas requiring the greatest need of conservation to prevent imminent global extinctions (Ricketts *et al.* 2005).

On the Red List of Threatened Species published by the International Union for Conservation of Nature (IUCN), *E. salvaje* was recently reassessed as Endangered B1ab(iii), because of its range restriction to two threat defined locations (<1000km$^2$ extent of occurrence) and continued decline in the extent and quality of forest habitat in southern Guatemala and northern Honduras (IUCN SSC Amphibian Specialist Group 2020). Though previously considered as Critically Endangered and endemic to Honduras (Wilson *et al.* 1985; Mendelson 2015; Solis *et al.* 2017), newly emerging as yet unpublished evidence indicates *E. salvaje* populations remain in once connected regions of suitable montane forest in southern Guatemala, i.e. Cerro del Mono, Zacapa, as well as potentially within the internationally recognised 'Reserva para la Conservación de Anfibios, Sierra de Caral' (C. Vásquez-Almazán pers.comm; IUCN SSC Amphibian Specialist Group 2020). While *E. salvaje* was initially documented in extreme eastern Guatemala (Wilson *et al.* 1985; Mendelson *et al.* 2008), the individual metamorph reported from that location is now referred to *Trachycephalus typhonius* (Mendelson *et al.* 2015).

To date, only four adult individuals of *E. salvaje* have been reported since its description by Wilson, McCranie & Williams (1985). The known specimens include an adult male holotype (1982), adult male paratype (1983) and two lots of tadpoles (seven described) collected alongside the former adult *E. salvaje* at the type locality, Quebrada Grande in Parque Nacional Cerro Azul Copán, Department of Copán (Wilson *et al.* 1985; McCranie & Wilson, 2002). In CNP (Departments of Cortes and Santa Barbara), one voucher photo of an adult was reported from the western region (camp El Cortecito) in 2009 (Kohler 2011; Mendelson *et al.* 2015; J. Kolby pers. comm) as well as one well-documented sub-adult from the east (camp Guanales) in 2014 (Solis *et al.* 2017; IUCN SSC Amphibian Specialist Group 2020). It has been speculated that *E. salvaje* may have been extirpated from its Honduran type locality in Copán owing to severe habitat degradation (J.M Solis and F. Castañeda pers. comm; IUCN SSC Amphibian Specialist Group 2020), however focused surveying of this region is needed to determine whether populations of *E. salvaje* persist within the remaining forests.

During field expeditions to CNP in 2017 and 2018, we encountered four individuals of *E. salvaje* at a single forest locality named Guanales, CNP; in the same region as the specimen collected by Solis *et al.* (2017). Herein we present the morphometrics, chytrid sampling data and behavioural observations concerning each of our *E. salvaje* encounters in the order they were made, with accounts of two records from 2009 and 2014, and lastly, discussing our results on morphology, behaviour, ecology, and conservation implications.

# Methods

## Study Site

CNP is a relatively small, protected area in north-western Honduras encompassing a portion of the Omoa Mountains. It was originally established as a national park due to the necessity of the Honduran government to protect crucial water sources for the nearest city, San Pedro Sula. CNP consists of two major protected areas; a 7,690 ha core zone where agriculture and settlements are not permitted, and a 15,750 ha buffer zone which allows for restricted land-use and anthropogenic activity (Hoskins *et al.* 2018). Operation Wallacea has been running long-term biodiversity monitoring in CNP for over 15 years utilising a network of temporary research camps throughout both the core and buffer zones (Gilroy *et al.* 2017; Martin *et al.* 2021). Both prior records of *E. salvaje* in CNP have occurred in the vicinity of two research camps (Guanales and El Cortecito - IUCN SSC Amphibian Specialist Group 2020). The habitat at Guanales and El Cortecito is similar, characterised by pristine lower montane broadleaf forest at approximately 1287 - 1400 m a.s.l., with both camps situated on the side of a valley <50 m from the closest stream. The microclimate is humid with frequent rainfall and cool temperature (Guanales, Cortecito: mean annual precipitation 1726, 1793 mm, mean annual temperature 19.9, 19.0 Celsius (O. Burdekin. pers. comm.)). At these elevations in CNP, there is an increasing lowland rainforest element in the forest flora, represented among the trees by families such as Annonaceae, Moraceae and Vochysiaceae (S. Batke. pers.comm). The understory vegetation is lush and leafy, composed of broad-leaf saplings, palms, orchids, climbing epiphytes and large tank forming bromeliads. Large mature trees were noted to provide hollows or cavities which collect water, considered an essential habitat component for the reproduction of *E. salvaje* at these localities (Wilson *et al.* 1985).

## Data Collection

Observations by the authors were made during the months of June through August 2009, 2014, 2017, and 2018. Upon encountering *E. salvaje*, we noted the date and time, and took comprehensive details of the locality, habitat and immediate behaviour. All individuals were handled and processed using aseptic techniques, before collecting chytrid swab samples in accordance with protocols established by Hyatt *et al.* (2007). After swabbing, we measured the snout-vent length (SVL) of all individuals in millimetres (mm) using digital callipers and placed them in a clear plastic bag to measure body mass in grams (g) using an appropriate Pesola scale. Using digital callipers to the nearest mm, where possible, we collected detailed morphometric measurements on the length of the humerus, femur, radius and tibia, as well as head length, head width, inter-tympanum and intraocular width. For adults, we determined sex of males based on the presence of large, spiny clavicle protuberances, short robust humerus (Wilson, McCranie & Williams 1985) and a strongly projecting prepollex bearing black nuptial excrescences (Mendelson *et al.* 2015); sexually dimorphic characteristics are apparently reduced or absent in females. For metamorphosing tadpoles, we also measured tail length in mm. Stage of tadpole metamorphosis was determined using the characteristics outlined in Gosner (1960). Modal webbing formula (Savage and Heyer 1967) was recorded for the two individuals found by the authors in 2018. Roman numerals denote digits, subscript Arabic numerals denote the intercalary and subarticular tubercles, distal to the webbing, and their respective + or - demarcate when webbing reaches the proximal or distal margin of these tubercules respectively. All individuals were photographed and released back at their capture sites within 24 hrs. Due to any conservation risk associated with this rare and charismatic species, we chose not to collect scientific specimens, nor to report GPS coordinates for our encounters herein. The exact location data is catalogued in the Operation Wallacea database.

## Chytrid sampling

Following Hyatt *et al.* (2007), we used sterile cotton-tipped swabs to sample the ventral surfaces of the limbs and drink patch 5 times each, applying moderate friction whilst rotating the swab bud. Swab buds were then snapped off and stored in 2 ml screw-capped vials containing 500 µl of preservative ethanol (99%), as high relative humidity in CNP prevents alternative preservation methods like air drying (Kolby *et al.* 2010). Samples were kept refrigerated until export, after which they were stored at -20 °C until DNA extraction.

Prior to DNA extraction, swab samples were centrifuged at 8,000 rpm for 30 min to pellet any particulate matter present in the preservative ethanol. Ethanol was then aspirated from the samples using a micropipette and residual ethanol was dried by placing the samples in a heat block under a laminar flow hood. Genomic DNA was then extracted from skin swabs using the DNeasy Blood and Tissue kit (Qiagen) according to manufacturer's instructions and eluted to a final volume of 100 µl.

*Bd* infection status and load was quantified from extracted DNA using a *Bd* specific quantitative polymerase chain reaction (qPCR) assay targeting a portion of the ITS-1/5.8S ribosomal gene region as described by Boyle *et al.* (2004) and Hyatt *et al.* (2007) run on a CFX96 real-time system (Bio-Rad). Extracted DNA samples were diluted 1:10 in laboratory grade distilled water, and bovine serum albumin was added to the qPCR master mix to reduce any potential PCR inhibition (Garland *et al.* 2010). Positive controls of known concentration were used as Bd DNA standards (100, 10, 1, 0.1 Bd Zoospore Genomic Equivalents) as well as a no template negative control containing laboratory grade distilled water. All samples were run in duplicate and *Bd* load was quantified by comparing sample qPCR values to those obtained from DNA standards (implemented in R), these were then corrected for the dilution factor and converted to genomic equivalents (GE) per swab. Samples were considered positive if amplification occurred in both duplicates with a mean load greater than 0.1 GE.

# Results

## *Observations and morphology*

At c. 20:30h on 28 July 2017, an *E. salvaje* metamorph was encountered and photographed in the late stages of development (Figure 1 a, b), exhibiting features consistent with Gosner tadpole stage 45 (Gosner 1960). The individual measured 22 mm SVL, weighed 2 g, and had a short 2 mm tail present that was in the process of absorption (Table 1). Few annotations of its behaviour or ecology can be documented, as the *E. salvaje* in question appeared unexpectedly on a notepad used for recording data. Whether it jumped from the adjacent leaf litter or fell directly from the canopy is unknown, although the latter may be most plausible as *Ecnomiohyla* species are usually canopy dwelling (Mendelson *et al.* 2015). It should be noted that we were initially unable to identify this metamorph as *E. salvaje* in the field, because of our remoteness and limited access to relevant literature. However, it was immediately acknowledged to be different from more commonly observed sympatric species, e.g. *Plectrohyla, Smilisca,* etc. Through subsequent analysis of the photographs taken, we matched the phenotypic morphology present in this Gosner stage 45 metamorph to those tadpole specimens in Gosner stage 34, 36, 40, 41, 42 and 43, described by Wilson *et al.* (1985). Clear similarities are present such as extensive webbing, dorsal tubercles, two black bands on the hindleg and the early development of white scalloped dermal fringing along the outer edge of the forearm and fourth finger as well as the outer edge of the foot and fifth toe (Figure 1 a, b).

Our initial presumptions were corroborated when an adult *E. salvaje* was found at the same location two nights later, on 30 July 2017, at 21:00h. Upon returning to the site, a large frog was spotted about 5 m above the ground on the branch of a tree, perched ca. <2 m from a large tree hole (Figure 2 b). To confirm the observation, we attempted to retrieve the individual from its perch carefully using a long branch. When disturbed, the individual jumped ca. 3 m to another branch, and was subsequently captured. This adult female measured 70 mm SVL and weighed 53 g (Table 1). During handling, the female *E. salvaje* proceeded to inflate its body in defence (Figure 3 a, b) and within minutes its colouration changed from a mottled mossy green to a dark reddish-autumn brown, as shown here 90 mins after capture (Figure 3 c).

In 2018, we revisited the locality (Figure 2 a, b) to search for *E. salvaje*, which yielded observations of a second metamorph (Figure 1 c, d) and an adult male (Figure 3 d). On 30 June 2018 at 21:00h, the second metamorph (Gosner stage 45 tadpole) was found sitting on low vegetation directly under the same tree where the previous individuals were found. When encountered, the metamorph was in a resting position with its head lowered and appendages closely tucked against its body. The individual exhibited pale grey dermal pigmentation interspersed subtly with iridescent cyan-teal green colouration and darker veinlike patterns (Figure 1 c); within minutes of capture the individuals' colour turned much darker owing to metachrosis, as shown here 24hrs later on release (Figure 1 d). The metamorph had an SVL of 24 mm, a body mass of 1.1 g, and residual tail length of 2 mm (Table 1). Much like the 2017 metamorph (Figure 1 a, b), the 2018 individual (Figure 1 c, d) possessed two clear dark banding patterns on the hind limbs, white scalloped dermal fringing, indiscriminate lateral banding across the fingers and toes, orange-tinted toe-disks, interwebbing and inner thigh-flanks, a distinctive dark circular patch above the forehands, a dark band beneath the eye, dark smudging on the tympanum, and venose dark unconnected patterns present on the forehead, dorsum and dorsolateral flank. When the appendages were tucked in a resting position, the banding on the forearm was adjoined with dark smudges beneath the eye and tympanum to blend and better disguise its appearance (Figure 1 c).

On 28 June 2018 at 20:25h, an adult was spotted sitting inside the tree hollow, ca. 2 m adjacent to

TABLE 1. Morphology of the four individual Ecnomiohyla salvaje found in 2017-2018

| Lifestage | Sex | Mass (g) | Snout Vent Length (mm) | Tail Length (mm) | Humerus (mm) | Femur (mm) | Radius (mm) | Tibia (mm) | Bd Genomic Equivalents (GE) |
|---|---|---|---|---|---|---|---|---|---|
| Adult | F | 53 | 70 | - | | | | | 0.24 |
| Adult | M | 39.6 | 79 | - | 11.25 | 36.5 | 21.5 | 42.75 | 1.22 |
| Tadpole Stage 45 (2017) | U | 2 | 22 | 2 | | | | | 53.96 |
| Tadpole Stage 45 (2018) | U | 1.1 | 24 | 2 | 3.5 | 11.5 | 5.75 | 7.5 | 2.20 |

Table 1. Morphology of the four individual *Ecnomiohyla salvaje found* in 2017-2018: - data not collected; F, Female; M, Male; U, Unknown; All body measurements are in grams or millimetres; skeletal measurements are provided as an average between respective limbs on both sides of the body. qPCR test results for *Bd* infection loads are given for each individual in zoospore genomic equivalents (GE).

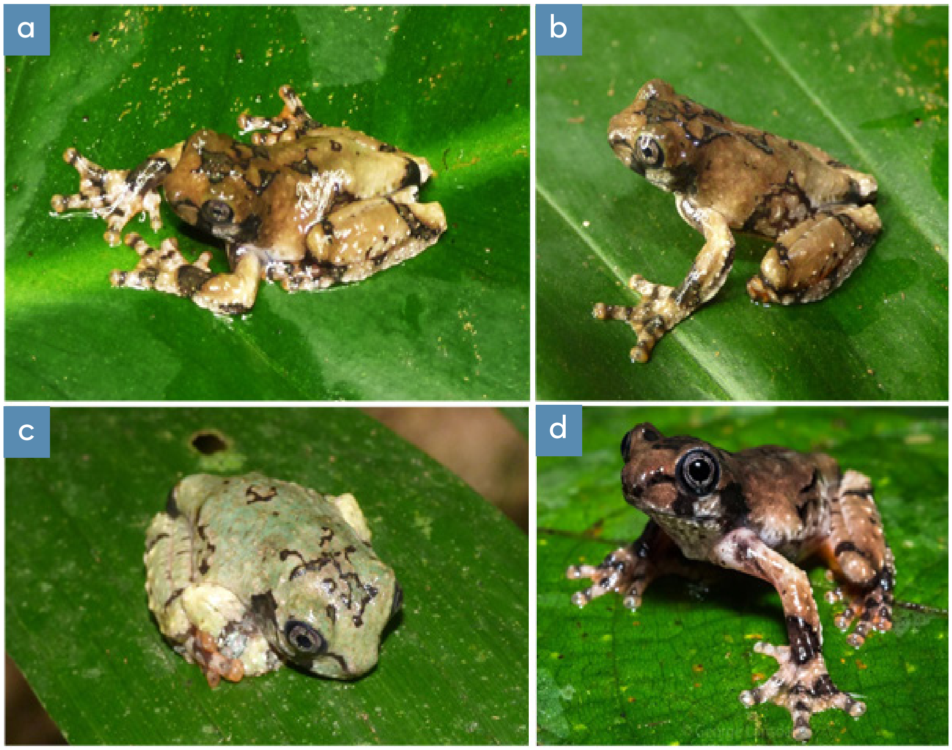

**Fig. 1** Photographs of recently metamorphosed *E. salvaje*; Gosner stage 45 tadpoles. **A)** Dorsal view of 2017 metamorph showing marbled brown-black colouration and well defined webbing on forehands with orange tints. **B)** Lateral view showing body profile, the development of dermal tubercles and white scalloped fringes on arms and legs. **C)** The 2018 metamorph pictured in-situ as encountered, exhibiting lighter silver and green dorsal colouration with appendages tucked besides body. **D)** The 2018 metamorph pictured upon release c. 24hrs later, exhibiting darker dorsal colouration post metachrosis. © (A, B, C) Cameron Thorp, (D) George Lonsdale

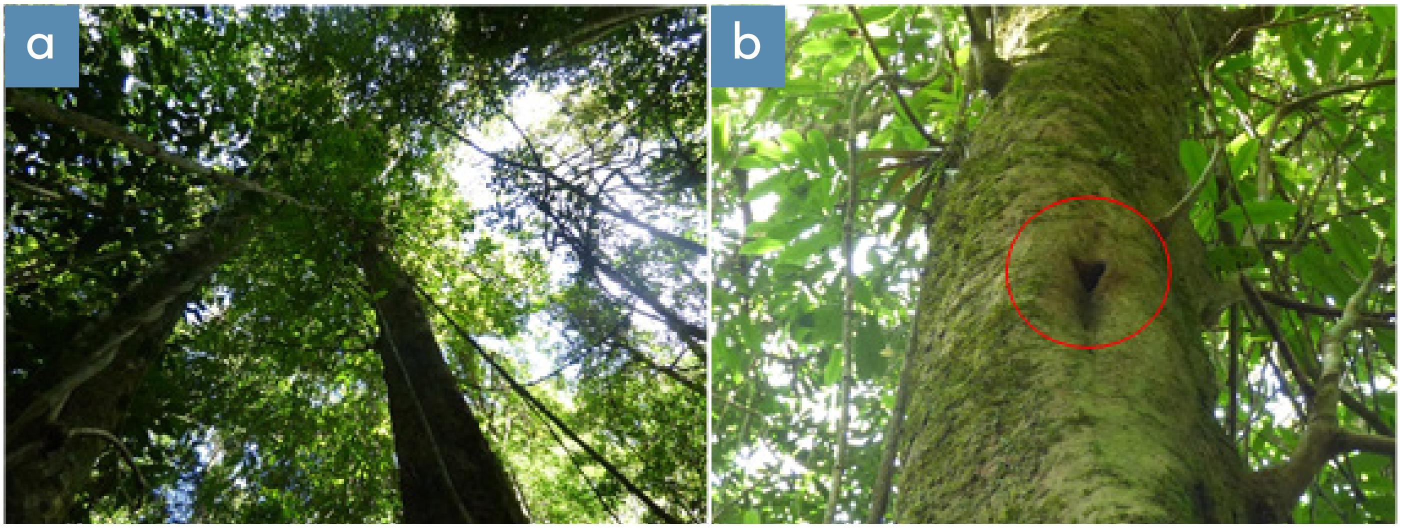

**Fig. 2** Site of encounter and assumed maternity tree for both metamorph and adult *E. salvaje* in 2017 and 2018, located in lower montane broadleaf forest, Guanales, CNP. **A)** Indicates the height and density of the canopy above the tree; **B)** the location of the tree hollow (circled red) which adults were observed utilising; Gosner stage 45 tadpoles were found beneath this tree. © (A, B) Cameron Thorp

where the adult female was observed in 2017, but we were unable to catch it. Three nights later, on 1 July 2018, at 20:00h, we returned and observed, assumedly, the same adult on a branch close to the tree hole, from which we were then able to successfully capture it. The adult male had an SVL of 79 mm and body mass of 39.6 g; additional detailed morphometric data was taken of key body dimensions and external head morphology (Table 1 & 2). We determined this individual

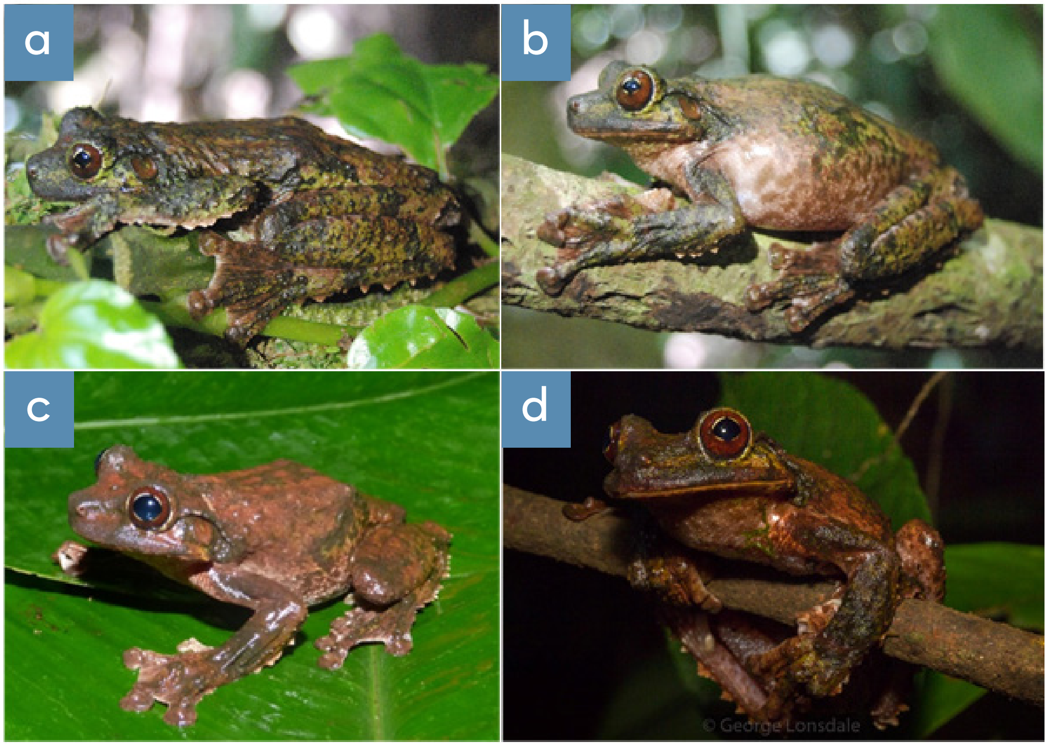

**Fig. 3** Adult *E. salvaje* referenced in the text: A) female encountered in 2017 exhibiting a mottled mossy green colouration when found; B) same female as A inflating its body as an anti-predatory defence after being handled; C) same female ca. 90 mins after capture, showing colour change to a dark rusty-reddish brown following metachrosis. D) Male encountered in 2018 perched on a branch. © (A, B) Cristina Arrivillaga; (C) Cameron Thorp; (D) George Lonsdale

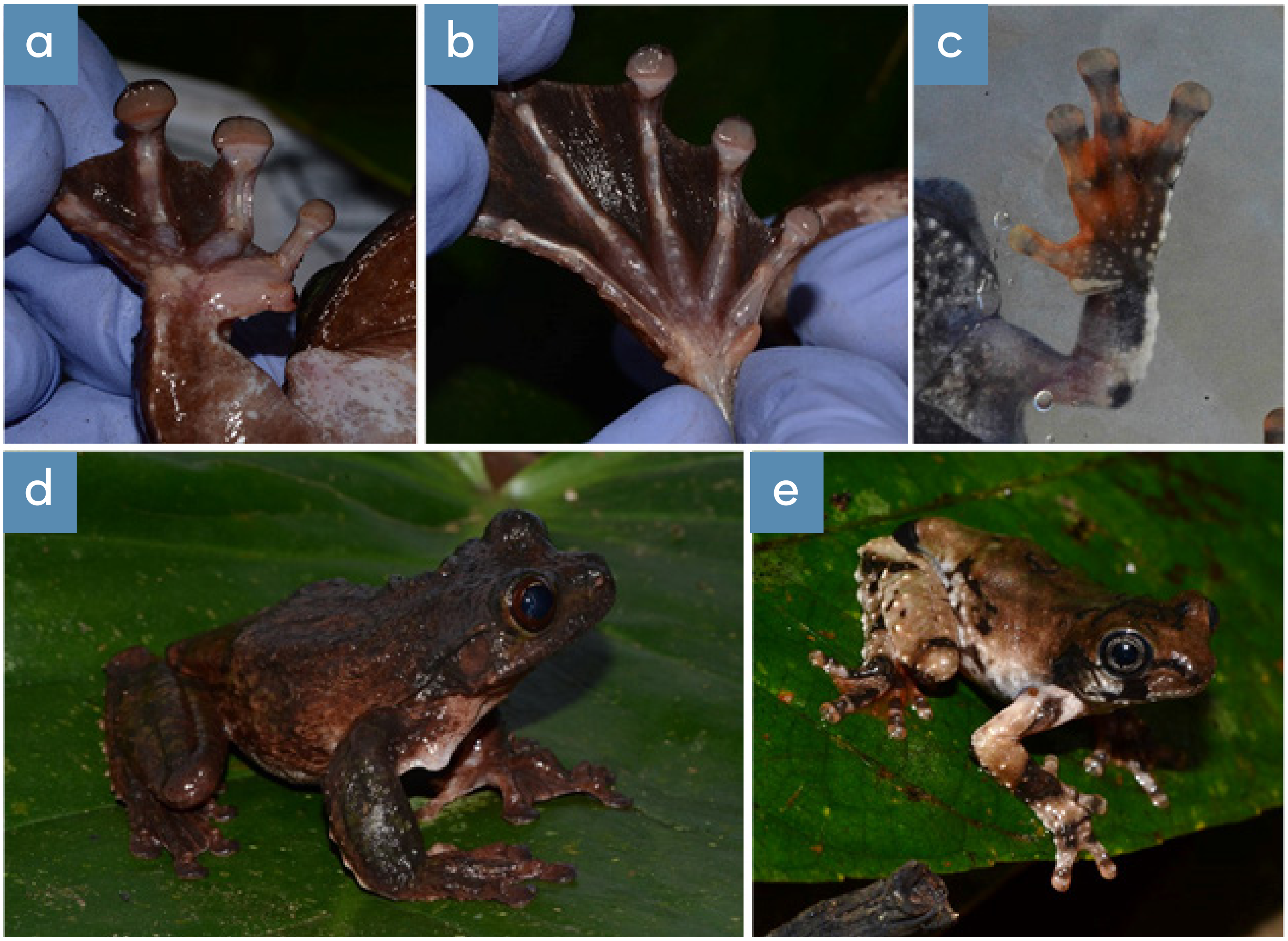

**Fig. 4** Additional images of key diagnostic features. A) The right hand of the 2018 male *E. salvaje* I $_{2}^{-}{}_{-2}^{+}$ II $_{1\text{-}1}^{+}$ III $_{1}^{-}{}_{-1}$ IV (D) exhibited well developed interdigital webbing, expanded toe-disks and a strongly projecting prepollex with cluster of black excrescences on outer edge. B) The right foot webbing of male E. salvaje (D) I $_{1\text{-}2}^{+}$ II $_{0}^{+}{}_{-2}$ III $_{0}^{+}{}_{-1}^{-}$ IV $_{1}^{-}{}_{-1}^{-}$ V, C) The forehand of the 2018 metamorph (E) (finger webbing I $_{2}^{-}{}_{-3}$ II $_{1}^{+}{}_{-2}^{+}$ III $_{2}^{-}{}_{-2}^{-}$ IV; toe webbing I $_{1}^{+}{}_{-2}^{+}$ II $_{0\text{-}2}$ III $_{1}^{+}{}_{-2}^{-}$ IV $_{2}^{-}{}_{-1}^{+}$ V) had bright orange interdigital webbing with banding across digits, and the development of white scalloped dermal fringes on the fourth finger and outer forearm. D) The 2018 male E. salvaje turned a dark colouration during handling; note the prominent 'spine-like' clavicle protrusion; absent from the 2017 female. E) The 2018 metamorph pictured. © John David Curlis.

(Figure 3 d) was a different adult to that previously captured in 2017 (Figure 3 a, b, c) from the same tree on the basis of sex and morphology. Defensive inflation behaviours and metachrosis were also observed with this individual, during and after handling.

The adult and metamorph *E. salvaje* found in 2018 had morphological measurements recorded, including the modal webbing formula. There were some slight differences between individuals. The metamorph *E. salvaje* had finger webbing I $_{2}^{-}{}_{-3}$ II $_{1}^{+}{}_{-2}^{+}$ III $_{2}^{-}{}_{-2}^{-}$ IV (Figure 4 c, e) and toe webbing I $_{1}^{+}{}_{-2}^{+}$ II $_{0\text{-}2}$ III $_{1}^{+}{}_{-2}^{-}$ IV $_{2}^{-}{}_{-1}^{+}$ V. The adult male had finger webbing I $_{2}^{-}{}_{-2}^{+}$ II $_{1\text{-}1}^{+}$ III $_{1}^{-}{}_{-1}$ IV and toe webbing I $_{1\text{-}2}^{+}$ II $_{0}^{+}{}_{-2}$ III $_{0}^{+}{}_{-1}^{-}$ IV $_{1}^{-}{}_{-1}^{-}$ V (Figure 4 a, b, d).

## *Presence of Chytrid (Bd)*

Swabs collected from the four *E. salvaje* individuals all tested positive using qPCR for the presence of *Bd* with infection loads ranging 0.24 - 53.96 GE (Table

TABLE 2. External head morphology of the two individual Ecnomiohyla salvaje found during June 2018.

| | Sex | Head Length (mm) | Head Width (mm) | Inter-Tympanic width (mm) | Intra-Ocular width (mm) |
|---|---|---|---|---|---|
| Tadpole Stage 45 (2018) | U | 8 | 8.5 | 8 | 3 |
| Adult | M | 28.5 | 31.5 | 34 | 10 |

**Table 2.** External head morphology of the two individual *Ecnomiohyla salvaje* found during June 2018. Head length is measured from the posterior corner of the mouth to the most distal point of the snout; Head width is measured between the two most proximal points of the tympanic membranes; Intraocular width is the distance between the upper eyelids at their most proximal points. All morphological measurements are in millimetres.

1). Each adult displayed a lower infection load than each metamorph swabbed within the same season. The highest infection load (53.96 GE) was harboured by the 2017 metamorph, whereas the 2017 adult female approached the lower threshold for positive diagnosis used in the current study (0.1 GE). The 2018 adult male and 2018 metamorph displayed a much lower range of variation at 1.22 and 2.2 GE respectively.

### *Additional records*

Lastly, for the purpose of accounting for all known individuals, we add one unvouchered record of *E. salvaje* to the known total of adult individuals encountered in CNP prior to 2017. On 26 July 2014, an adult *E. salvaje* was encountered at Guanales during the day, resting on a horizontal mossy tree branch c. 1.5 m high alongside a stream in lower montane forest habitat. The individual was captured by Julius 'Tony' Frazier (T.W. Brown pers. comm.) and known voucher photographs were taken separately by Achyuthan Srikanthan (Figure 5 a & b). Moreover, we formally catalogue additional voucher photographs and clarify details for the existing 2009 adult specimen from El Cortecito, 1355 m a.s.l., known by J. Kolby pers. comm (IUCN SSC Amphibian Specialist Group, 2019), pictured in Kohler (2011 Fig. 538) and discussed by Mendelson *et al*. (2015), which actually refers to the first ever *E. salvaje* found in CNP, encountered by Andrew Snyder (Figure 5 c & d). To expand on the few details associated with that record, the latter adult male individual was encountered on July 26, 2009, at ca. 20:30h along the ridgeline near El Cortecito. The individual was perched on the flagging tape used to demarcate a transect wrapped around the trunk of a tree approximately 1 m off the ground. However, due to possible human error with CNP data transfer and input during those years, the morphological and swab data associated with both of these prior records could not be located for our present study. Despite the apparent absence of basic data, we count them to be valid additional observations of *E. salvaje* in CNP.

## Discussion

### Morphology

Our morphological results for an adult male *E. salvaje* (79 mm SVL) were consistent with the maximum recorded size for males, <86 mm SVL (Wilson *et al*. 1985). Our data collected on male tibia length [TL]

**Fig. 5** Additional voucher records of *E. salvaje* from CNP, for which morphological data is unavailable. A & B) An unsexed adult found in 2014 at Guanales, posed on a branch (A) and resting on a broad leaf (B); mossy body colouration and tucked appendages with scalloped dermal fringes provide excellent camouflage. C & D) The prior referenced adult male individual encountered in 2009 at El Cortecito; the pronounced clavicle protrusion is clearly visible (D). © (A, B) Achyuthan N. Srikanthan, (C, D) Andrew Snyder

(42.75 mm [54.1% of SVL]) however, was slightly greater than the respective holotype and paratype male (TL 41- 44.6 mm [48.4- 51.9% of SVL] - Wilson *et al.* 1985). Head width (31.5 mm) and head length (28.5 mm) were also consistent (± 2 mm) with the respective 31.3 mm and 28.7 mm listed by Mendelson *et al.* (2015). Our measurement of the first adult female (SVL 70 mm), may suggest that females are smaller than males; consistent with male-bias sexual size dimorphism exhibited throughout the *Ecnomiohyla* genus (Mendelson *et al.* 2015). While we unfortunately did not collect detailed morphological measurements of the female, it was noted to have reduced musculature around the upper forearms than those of the male. Furthermore, the 2018 adult male found by the authors, exhibited large spiny clavicle protuberances (Figure 4 d). This, in combination with the presence of other secondary sexual characteristics i.e. a large projecting prepollex with black nuptial excrescences in a crescent shaped cluster (Mendelson *et al.* 2015; Figure 4 a), confirmed this individual as a male. The female was identified by the absence of large spiny clavicle protuberances, as well as having

a less pronounced prepollex lacking black nuptial excrescences.

Extensivity of webbing in hylid frogs is variable and is a commonly used trait to characterise different species. The modal webbing formula of the two individuals found in 2018 differed slightly from that given in Mendelson *et al.* (2015) and the basic description from the species authority (Wilson *et al.* 1985). The slight variation here is likely due to the respective small sample sizes. However, if more individuals are found at the type locality, CNP or in other regions then care should be taken to record this feature. When combined with genetic techniques, this could shed light on the species' evolution and phylogeny.

## Ecology and defensive behaviours

Our behavioural observations for adult individuals are mostly consistent with those reported for other members of the genus. Frogs belonging to the genus *Ecnomiohyla* are known to glide or parachute using their extensive dermal webbing (Mendelson *et al.* 2008), and although this was not explicitly observed, we did observe the adults jumping large distances (c. >3 m), with their substantial webbing presumably helping direct and maintain such leaps within the canopy. Additionally, metachrosis (green to brown colour change) had also been previously documented in *Ecnomiohyla* (Mendelson *et al.* 2015), suggesting that colouration may not be a reliable diagnostic character for these frogs (Savage & Kubicki, 2010; Kubicki & Salazar, 2015). Several environmental factors have been found to cause metachrosis in anurans, including temperature, humidity, background, communication, and stress (King *et al.* 1994). We suggest stress as the most likely cause for the darker colour change seen in our captured *E. salvaje;* yet, considering the extreme adaptive lengths *E. salvaje* has gone to otherwise conceal its body, metachrosis may play an active role in camouflage. Based on the observed metamorphs, *E. salvaje* appears to undergo a degree of ontogenetic colour change. Additionally, while the camouflage of adult *E. salvaje* gives the appearance of bark or moss, metamorph colouration gives the appearance of a bird dropping, a form of phaneric mimesis seen in other anurans (i.e. *Dendropsophus marmoratus* and *Theloderma* spp.) and suggests they may masquerade in plain sight while sitting on leaf surfaces (Toledo & Haddad, 2009). We also observed that while handling, both adult male and female individuals very clearly began to inflate themselves and 'puff up,' a common defence mechanism in other hylids (Toledo *et al.* 2011; Escobar-Lasso & González-Duran, 2012). In large-sized hylids, inflation may be particularly effective as it renders them more challenging for predators to subdue (Toledo *et al.* 2011; Arrivillaga & Levac, 2019). Although we did not explicitly observe it in *E. salvaje*, a secondary defense associated with bloating mechanisms is phragmosis (Toledo *et al.* 2011), whereby the individual will inflate and wedge its body or head tightly within the cavities of trees to block the entrance from predators or improve water economy (Jared *et al.* 2005); this behaviour is exhibited almost exclusively in hylids with co-ossified skulls. *Ecnomiohyla* species depend on tree cavities due to their lifestyle and reproduction, and several species, including *E. salvaje*, have a co-ossified skull (Mendelson *et al.* 2015; Solis *et al.* 2017). Therefore, we suggest it is probable that defensive inflation is used in tandem with phragmotic 'tree crevice-obstruction' behaviours, as when two or more defensive strategies are used concomitantly, the net benefit is usually higher than the simple sum of effective isolated behaviours (Toledo *et al.* 2011).

Furthermore, our observations of two metamorphs, an adult female and male in close proximity to a tree hollow provides more support that the genus *Ecnomiohyla* utilises tree holes in various different ways, including for refuge, as breeding sites (Savage & Kubicki, 2010) and as a place for tadpoles to metamorphose into adulthood (Wilson *et al.* 1985; Mendelson *et al.* 2008). In *E. rabborum,* clutches of 60 - 200 eggs are deposited just above the water line on the exposed wood or bark inside tree holes; the males remain at the hole in attendance of eggs and tadpoles, while females disperse soon after oviposition (Mendelson *et al.* 2008). Much like its congener, the male holotype and paratype *E. salvaje* were collected while in attendance of tree holes containing tadpoles (Wilson *et al.* 1985); our observations of adult and metamorph *E. salvaje* present around the tree hole also support that some element of parental care may occur. In our study, despite being 9 mm larger in SVL, the male was 12.4 g lighter than the female, which perhaps suggests that the female was gravid. While this was not confirmed, this hypothesis is supported in most species of *Ecnomiohyla*, wherein females are attracted by males to breeding holes solely to reproduce while males remain at the breeding site to attend eggs and tadpoles (Wilson *et al.* 1985; Mendelson *et al.* 2008). However, it has been suggested that tadpoles of some species (e.g. *E. miliaria*) may consume eggs deposited by the mother (Kohler, 2011; Mendelson *et al.* 2015), which implies they too may assist in parental care. Generally however, it is believed males are more responsible for tadpole attendance. In captivity, *E. rabborum* tadpoles have also been observed eating skin flakes from an attending male sitting half-submerged in the water (Mendelson *et al.* 2008). Male care and paternal duties correlate with the adaptation of male-bias sexual size dimorphism in other anurans (Vági *et al.* 2019), and we suggest this could be a driving factor for the greater maximum male size in members of the *Ecnomiohyla* genus, such as *E. salvaje.*

The two metamorphs we describe differ from those seven free-swimming tadpoles described by Wilson *et al.* (1985), given both were in late stages of metamorphosis, active at low height, and no longer dependent on the water of tree cavities. Both metamorphs (2017 and 2018) were encountered within 10 m of the same tree cavity, frequented by adults for at least two consecutive years, which is presumably where those offspring developed. We suggest *E. salvaje* may repeatedly use the same hollows to reproduce, perhaps because mature trees with trunks providing appropriate water-filled cavities for breeding are uncommon, and so adults (especially males) are fidelitous to suitable reproductive territory. The presence of separate late-stage metamorphs both in the leaf litter and on low vegetation is unusual for canopy dwelling species, but suggests *E. salvaje* may leave their hollow shortly after metamorphosis. Whether they mature in mid-low vegetation or disperse rapidly into the canopy of neighbouring trees is unknown and is an area of interest for future studies.

## Conservation Implications

Numerous anthropogenic practices directly endanger *Ecnomiohyla* populations, including habitat loss and degradation by means of deforestation, forest fires, and expanding agriculture and development. Further confounding these is the threat posed by chytridiomycosis, a lethal epizootic disease of amphibians caused by *Batrachochytrium dendrobatidis* (*Bd*). *Bd* is a fungal pathogen which infects the keratinised tissues of the epidermis, impairing osmoregulatory function, and may cause skin lesions, hyperplasia, excessive sloughing and death (Berger *et al.* 2005; Marcum *et al.* 2010). Despite the fact that variation in susceptibility to *Bd* exists between and within species (Blaustein *et al.* 2005; Bradley *et al.* 2015), there is so far limited evidence of amphibian populations recovering from chytrid epidemics. To date, chytridiomycosis is recognised as being responsible for the greatest recorded loss of biodiversity attributable to a disease, having driven the declines of over 500 amphibian species and the extinction of more than 100 species, with only 60 species (12% of declined species) showing initial signs of recovery (Scheele *et al.* 2019). Extinctions attributable to *Bd* may have already occurred in the genus *Ecnomiohyla*. The Critically Endangered *E. rabborum* is feared to be extinct following the death of the last known adult individual in captivity at Atlanta Botanical Garden in 2016 (AmphibiaWeb, 2018; IUCN SSC Amphibian Specialist Group 2019); previously a clutch of fertile eggs was reared *ex situ*, but those offspring did not survive. The dramatic decline of *E. rabborum* throughout its natural habitat was associated with the regional spread of *Bd* (Mendelson *et al.* 2008), as well as increased deforestation and anthropic disturbance. Recent searches have failed to locate surviving populations (IUCN SSC Amphibian Specialist Group 2019), but conservationists remain hopeful that additional wild populations do still exist. It is likely that *E. salvaje* is also highly susceptible to chytridiomycosis, but until our current study, no individuals had been tested.

Previous studies (Kolby *et al.* 2010; 2015a,b; Blooi *et al.* 2017) have recorded the presence of *Bd* in seven anuran species across three families from CNP, while also identifying various previously undocumented pathways for terrestrial and aerial dispersal of the disease. These findings attest to the pervasiveness of *Bd* as a pathogen and its ability to threaten an ecologically diverse range of species. Despite *E. salvaje* not being associated with 'traditional' sites for *Bd* transmission i.e. streams, pools and bromeliads, there is potential for *Bd* transmission into canopy environments through residue left on vegetation from other sympatric amphibian species that move between aquatic and terrestrial habitats (Kolby *et al.* 2015a), as well as potentially being transmitted by wind, waterfall spray and rainfall (Kolby *et al.* 2015b). Studies outside of CNP have suggested that canopy-dwelling frogs may act as vectors for *Bd* (Stuckert *et al.* 2009; Lindquist *et al.* 2011), and that the damp arboreal refuges they inhabit (e.g. bromeliads) might serve as chytrid reservoirs (Cossel & Lindquist, 2009).

The positive test results for *Bd* in all four sampled *E. salvaje* adds further support for conservation of this species. However, extreme care should be taken when extrapolating results from a small number of individuals without accurate estimates of key parameters at the population level (e.g. size, trend, demography, *Bd* prevalence and load). We are unable to confirm whether the positive results in the current study represent an active *Bd* driven decline, however, this limited data presumably deriving from a single reproduction site demonstrates that *E. salvaje* are susceptible to infection and may experience high environmental exposure to *Bd* via tree-hole reservoirs.

In addition to the secondary threats of disease, amphibians in CNP are primarily threatened by increasing rates of deforestation and disturbance within the core zone of the park (Martin *et al.* 2021). *E. salvaje* is especially threatened, given that six of the seven individuals known from CNP (four adults and two tadpoles) are from a single location (Guanales) in the core zone. The second locality in the core zone (El Cortecito) where *E. salvaje* was originally found in 2009, has since been subject to increasing deforestation rates and disturbance, to such an extent that now much of the primary forest habitat in the immediate area is heavily degraded and/or lost.

Considering the apparent sudden decline and possible extinction of its congener *E. rabborum* is associated with chytridiomycosis and habitat disturbance (IUCN SSC Amphibian Specialist Group, 2019), we suggest that immediate precautionary actions be taken to establish an in-country captive assurance population of *E. salvaje* to safeguard, further study and hopefully breed the species for reintroduction to the wild. Such *ex situ* conservation efforts could be performed in collaboration with pioneering head-start projects such as at the Honduran Amphibian Research & Conservation Centre (HARCC), which is already intending to boost native populations of other critically endangered frogs in CNP by rearing tadpoles/metamorphs in captivity prior to their reintroduction as stronger adults (Kolby, 2013). Whilst the effects of chytridiomycosis can be mitigated in captivity, any post-*Bd* survival outside of a terrarium requires the existence of appropriate habitat for species reintroduction, therefore respective *ex situ* and *in situ* efforts to conserve *E. salvaje* and its habitat should be deployed in tandem to ensure that the remaining wild population and forest in CNP is protected. *Ex situ* initiatives should also be combined with *in situ* bioacoustic and focused canopy surveys in CNP, in order to determine the true abundance and distribution of *E. salvaje* as well as estimate its population trajectory in the immediate to long-term future (IUCN SSC Amphibian Specialist Group, 2020).

## Conclusion

Our reports of *E. salvaje* individuals from CNP add further support to the recent publication by Solis *et al.* (2017) concerning the park's importance for the conservation of threatened amphibians. Following a combination of personal accounts from senior staff, herpetofaunal research spanning the last fifteen years of Operation Wallacea expeditions in CNP (Martin *et al.* 2021), and a comprehensive scientific literature review, we believe our collective observations represent the first known encounters of *E. salvaje* metamorphs, and a 75% increase in the current total adult records ever known since the species description in Copán ca. 35 years ago (Wilson *et al.* 1985; Solis *et al.* 2017). As it stands, the total known *E. salvaje* observations now equate to seven individual 'adults', notwithstanding at least seven tadpoles and two recent metamorphs in various Gosner stages encountered on four separate occasions. In addition to providing valuable information on the morphology, defensive behaviour and ecology of *E. salvaje*, the fact so few observations have been generated despite many years of active surveying further attests to the necessity of running long-term biodiversity surveys to compile and maintain accurate species inventories in diverse cloud forest regions (Martin *et al.* 2021). It is hoped that the data we collated here will provide a valuable update and platform from which to expand the presently still limited information available on *E. salvaje*, in order to inform appropriate conservation action for the species.

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**Author Contributions:** All authors contributed equally to the writing of this manuscript and were involved with the Operation Wallacea field expeditions in CNP that led to the acquisition of these records. There are no conflicts of interest to declare.

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# Applying Species Distribution Models to identify areas of high conservation value for terrestrial mammals: A case study in Cusuco National Park, Honduras

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## Abstract

Terrestrial mammals are important ecosystem engineers in rainforest but threatened by deforestation and hunting even in areas with the highest level of designated protection e.g. National Parks. Species Distribution Models (SDMs) can be used to identify priority areas for vulnerable species informing the spatial allocation of conservation resources. Local SDMs do not rely on defining positive relationships that determine a species' optimal environmental niche but the negative relationships describing parochial species absence. Using species-specific field tracks and signs data from 2006 to 2017 for mammals in Cusuco National Park, Honduras, we built local SDMs for each species extrapolating the probability of occurrence throughout unsurveyed areas. We pooled predicted probabilities within species groups for i) low abundance, large-bodied, hunted species (e.g. tapir, deer or peccaries), ii) high abundance, small-bodied hunted species (e.g. paca or agouti) and iii) unhunted species (e.g. mustelids or wild cats). Results suggest that that despite the highest level of protected area designation local terrestrial mammal distribution was driven by local anthropogenic disturbance; specifically, deforestation within 2km, distance to the National Park boundary and distance to human populations i.e. proximity to villages (though not their population size), ecotourism camps and research stations. Local villages were ranked in descending order of their likely impact on large hunted mammals such that local Government departments, forestry managers, conservation organisations and patrolling wardens can use this information to target protection efforts (patrols, legislative enforcement and prosecutions) to maximise the efficacy of National Park designation and stem ongoing defaunation.

## Resumen extendido

Los mamíferos terrestres son importantes ingenieros de ecosistemas en la selva tropical, pero están amenazados por la deforestación y la caza incluso en áreas con el más alto nivel de protección designada, como en parques nacionales. El Parque Nacional Cusuco cubre 23,440 hectáreas de territorio protegido en la cordillera de Merendón, Cortés, noroeste de Honduras (15°32′31″ N, 88°15′49″ W). Está catalogada como la 123 área protegida más insustituible (48 considerando taxones amenazados solamente) a nivel mundial de los 173,000 sitios designados. Contiene bosque seco tropical de tierras bajas, bosque húmedo tropical, bosque nuboso montano y bosque enano localizado por encima de los 2.000 m sobre el nivel del mar. Tiene una rica diversidad de mamíferos terrestres (Hoskins *et al.* 2018); sin embargo, estos están altamente amenazados debido a la deforestación y la caza ilegal. Se prevé la destrucción total de la fauna del parque para mediados de la década de 2020 (Hoskins *et al.* 2020). Este estudio utilizó modelos de distribución de especies (SDM) para comprender los causantes antropogénicos locales que afectan los rangos de mamíferos dentro del Parque Nacional Cuscuo. El objetivo específico era analizar la presencia de especies en relación con los impactos humanos clave, como la proximidad a las rutas de acceso (por ejemplo, carreteras o pueblos) o el estado de protección (por ejemplo, el límite del Parque Nacional o las designaciones zonales internas), teniendo en cuenta a la vez los efectos de la variación ambiental natural en las variables bioclimáticas (por ejemplo, temperatura media anual y precipitaciones). Se planteó la hipótesis de que la probabilidad de presencia de mamíferos estaría asociada negativamente con perturbaciones antropogénicas.

Utilizando datos especie-específicos sobre signos y señales colectados en el campo desde 2006 a 2017, creamos SDMs a escala local para cada especie de mamífero para la cual había un tamaño de muestra suficiente mediante la extrapolación de la probabilidad de su ocurrencia en áreas no muestreadas.

Se agruparon las probabilidades pronosticadas dentro de los grupos de especies para I) especies cazadas de baja abundancia y de tamaño corporal grande (por ejemplo, tapir, venado o pecaríes), II) especies cazadas de alta abundancia y pequeño tamaño corporal (por ejemplo, paca o agouti) y III) especies no cazadas (ej. *mustélidos* o gatos salvajes). Utilizamos mapas de calor para trazar la probabilidad de ocurrencia. La probabilidad promedio de ocurrencia de especies de mamíferos dentro de 1 km de cada aldea dentro del Parque Nacional se clasificó en orden descendente para poder identificar aquellas aldeas con la probabilidad más baja (es decir, la mayor amenaza para los mamíferos terrestres).

Los SDMs locales no se basan en la definición de relaciones positivas que determinan el nicho ambiental óptimo de una especie, ya que no pueden capturar el rango completo de condiciones ambientales en todo el rango de cada especie, sino que las relaciones negativas que describen la ausencia de especies parroquiales. Nuestros resultados sugieren que, a pesar del nivel más alto de designación de área protegida, la distribución local de mamíferos terrestres ha sido causada por la perturbación antropogénica local; específicamente, por la deforestación dentro de los 2 km, la distancia al límite del Parque Nacional y la distancia a las poblaciones humanas, es decir, pueblos, campamentos de ecoturismo y estaciones de investigación. Las aldeas asociadas con la menor probabilidad de ocurrencia de grandes mamíferos cazados (por ejemplo, Santo Tomás y Corpus en el noroeste de la zona de amortiguamiento del parque, entre otros, como Regadio en el límite sur del parque) son similares a las actividades que representan la mayor amenaza para mamíferos terrestres por deforestación ilegal o caza. Los departamentos del gobierno local, los administradores forestales, las organizaciones de conservación y los guardias de patrullaje pueden usar esta información para enfocarse en los esfuerzos de protección (patrullas, cumplimiento de la ley y enjuiciamientos) para maximizar la eficacia de la designación del Parque Nacional y detener la continua destrucción de la fauna.

# Introduction

The 'Great American Interchange' of North American species dispersing south, and South American species dispersing north, after the formation of the Isthmus of Panama (Marshall *et al.* 1988), was responsible for the Central American Neotropics becoming one of the most diverse biogeographical realms on the planet (Myers *et al*., 2000). It is also one of the most vulnerable regions due to a combination of anthropogenic threats including; deforestation, hunting, road construction and climate change driven by human population growth and associated resource consumption (WWF 2018). Large-bodied, terrestrial mammals are particularly key in tropical forest function. They provide vital ecosystem services including; seed dispersal promoting forest regeneration (Fuzessy *et al*., 2016, Paolucci *et al.,* 2019), regulation of plant diversity and forest structure through herbivory (Wright *et al.,* 2000, Briceno-Mendez *et al*., 2016, Beck *et al*., 2013), top-down system regulation through predation (Marshall and Essington, 2011) and nutrient cycling via defecation and carcass decomposition (Sobral *et al*., 2017; Barton *et al*., 2013; Dos Santos Neves *et al*., 2010). Nutrient cycling promotes carbon sequestration (Wright *et al*., 2007) which is enhanced by terrestrial mammal diversity (Sobral *et al*., 2017). Just like the rainforests they inhabit, terrestrial mammals, particularly those with long-lives, slow reproduction and long maturity times, are notably vulnerable to local anthropogenic impacts, specifically deforestation, habitat fragmentation and hunting which leads to 'defaunation' i.e. forests mostly devoid of mammals (Redford, 1992, Wilkie *et al*., 2011, Benítez-López *et al,* 2019), and thus lacking their ecosystem service provision. Defaunation is most notable in close proximity to human settlements and access roads. Only 7% of global forest patches (>100km$^2$ in size) are free from the incursion of roads making most forests accessible for exploitation (Ibisch *et al*., 2016).

One of the key global, regional and local strategies for biodiversity conservation is the designation of protected areas, for example, National Parks

(IUCN and UNEP-WCMC, 2016; Tilman *et al*., 2017) within which potentially damaging anthropogenic activities are restricted or outlawed. Nevertheless, often due to a lack of clear evidence-based appraisal, there is generally a paucity of data by which to assess the efficacy of protected areas in preserving biodiversity (Geldmann *et al*., 2013). Global meta-analyses suggest that just 20-50% of protected areas are deemed to be 'well managed' and, therefore, by extension, to some degree effective (Watson *et al*., 2014). Thus, despite protected areas covering 14% of the planet (Butchart *et al*., 2015) there is concern over the high number of instances of poor ecological planning and management of such areas (Venter *et al*., 2014, Rodrigues *et al*., 2004, Butchart *et al*., 2015). Some suggest that conservation efforts have contributed little to offsetting global, regional and even local biodiversity loss and that legislative protection without enforcement frequently leads to so-called 'paper parks' (Joppa *et al*., 2008) were protective laws are unenforced and lack successful prosecutions of those that perpetrate damage (Joppa *et al*., 2008). Thus, understanding how terrestrial mammal distributions within Neotropical protected areas are impacted by local anthropogenic factors is important to focus conservation strategies under limited and nuanced human and fiscal resource availability (Oldekop *et al*., 2016).

Species Distribution Models (SDMs) use records for species occurrence coupled with spatially explicit environmental data to indicate the likelihood (probability) that unsurveyed areas are suitable for the species given known environmental tolerances (Elith & Leathwick, 2009). SDMs can be used in a number of ways; to better understand spatial ecology of rare or endangered species (e.g. Wilson *et al*. 2011), inform appropriate protected area designation (e.g. Chivers *et al*. 2013), understand risk from biological invasions (e.g. Kelly *et al*. 2014), better understand likely range shifts under predicted future climate conditions (e.g. Leach *et al*. 2015) or to contribute to our understanding of how biotic interactions limit species ranges (e.g. Leach *et al*. 2017). It is conventional and usually important to include all (or at least a large portion of) a species' total (global) range extent to ensure that the full variation of its environmental tolerances can be adequately captured (Araujo *et al*., 2019; e.g. Phillips *et al*., 2006). However, there have been studies where SDMs were used within a subsample of a species range (e.g. Angelieri *et al*., 2016 or El-Gabbas and Dormann, 2018). In isolated Neotropical forest fragments, it may be hypothesised that terrestrial mammal distribution may be spatially restricted principally by anthropogenic threats beyond species-specific tolerances of bioclimatic variation (Nagy-Reis *et al*., 2017). Thus, building spatially restricted SDMs may allow local drivers of distribution to be captured (e.g. Reino *et al*., 2018).

This study aimed to use SDMs to understand local anthropogenic drivers of mammal ranges within a highly biodiverse but isolated and threatened fragment of Neotropical cloud forest. The specific objective was to analyse species occurrences with respect to key human impacts such as proximity of access routes (e.g. roads or villages) or protection status (e.g. National Park boundary or internal zonal designation) whilst simultaneously accounting for natural environmental variation in bioclimatic variables (e.g. mean annual temperature or rainfall). It was hypothesised that the probability of mammal presence would be negatively associated with anthropogenic disturbance. Such responses are likely to be species-specific; we expected low abundance, large-bodied, hunted species (e.g. tapir, deer or peccaries) to exhibit stronger avoidance of human disturbance than high abundance, small-bodied hunted species (e.g. paca or agouti) which in turn were deemed more likely to respond than unhunted species (e.g. mustelids or wild cats). The purpose was to identify specific local regions of greatest potential human impact by ranking villages in order of the probability that they support terrestrial mammals in the surrounding landscape. Government departments, forestry managers, conservation organisations, legislative enforcement and patrolling wardens can use this information and spatially focus protection efforts to maximise the efficacy of National Park designation.

# Methods

## Study area

Parque Nacional Cusuco (hereafter, Cusuco National Park) is 23,440 hectares of protected land in the Sierra de Omoa of the Merendón mountain range, Cortés, north-west Honduras (15°32′31″N, 088°15′49″W; Figure 1). It is listed as the 123rd most irreplaceable (48th considering threatened taxa alone) protected area globally out of 173,000 designated sites (Le Saout *et al.*, 2013). The Honduran Government designated it under the Water Protection Act 87-87 in 1987 (ICF 2015). The Sierra de Omoa are isolated by the alluvial Sula valley to the east and Río Chamelecón valley to the south and southeast, the Río Motagua alluvial plain to the west and northwest and the Bahía de Omoa to the north. The park, a mountainous cloud forest region, rises to 2,240m above sea level, annual precipitation is 2,788mm, with mean monthly temperatures ranging from 13°C in December to 20°C in April (Fundación Ecologista 1994). There are four principal habitats within Cusuco National Park; i) tropical lowland dry forest, ii) tropical moist forest, iii) montane (cloud) forest and iv) 'bosque enano' (dwarf forest) occurring >2,000m.

The park has two levels of zonal protection; a core zone of 7,690 hectares surrounded by a buffer zone of 15,750ha. This zonal delineation occurred during 1994, designed by the Corporación Hondureña de Desarrollo Forestal (COHDEFOR), implementing a park management plan where in the 'core zone' agriculture, burning, mining, hunting, construction of roads, houses, or commercial, public and private institutions or any human settlements are not permitted (ICF 1987). In the 'buffer zone' construction of human settlements, excluding those that existed before the implementation of the management plan, are permitted but only under license as well as limited livestock grazing, burning, deforestation, mining, fishing, housing and road construction. Subsistence hunting may be permitted in this area but only if a permit is obtained, which requires travelling to San Pedro Sula, the regional administrative capital (a minimum of 4 hours travel away), and requires applicants to be literate, have an understanding of the relevant paper work and transport; consequently, bushmeat hunting is unlicensed and, therefore, illegal.

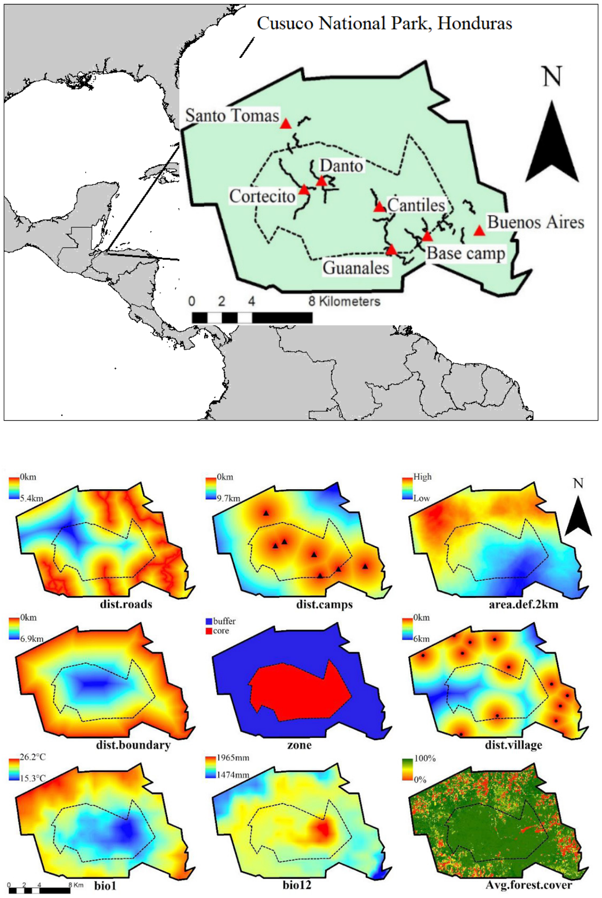


**Fig. 1** (a) Cusuco National Park, Honduras (15°32′31″N, 088°15′49″W). Red triangles = camps, black lines = transects, dotted line = buffer/core zone delineation. (b) Spatial variation in environmental parameters throughout the park

## Mammal surveys

Following long-term monitoring protocols across numerous taxa (plants, invertebrates, amphibians, reptiles, birds and mammals), over 12 years of species records have been collected (2006-2017) by Operation Wallacea Ltd. representing a network of research academics supported by student expedition fees which fund the programme (see www.opwall.com). Large mammal occurrence was surveyed annually (during June and July) using indirect field tracks and signs (i.e. spoor or footprints and/or faeces or other species-specific identification markers, for example, feeding signs, dens or direct sightings following Reid 1997; 2009). The rainy season along the north Caribbean coast of Honduras, including Cusuco National Park, runs from approximately November to March annually. Thus, data were collected in the middle of the dry season (with conditions being noticeably drier in more recent years in the time-series). Nevertheless, being predominately cloud forest, the region stays moist throughout the year. Surveys made use of a transect network consisting of 3-4 line transects 2-3km in length radiating out from seven research stations or camps (Figure 1a), although not all transects were used for the duration of the study period. Transects were walked at dawn (immediately after the nocturnal active periods of most species) with up to two repeat surveys of each transect per season. Due to various logistical issues (including the closing of the research station at Santo Tomas from 2014 onwards and aforementioned variation in transect use), survey effort (the number of repeated surveys of each transect) varied over time.

## Species records

Species were categorised into three groups; large hunted, small hunted and unhunted. Large hunted were those species identified as hunted by local people through interviews carried out in 2016 (H. Hoskins *unpublished data*) that on average weighed >2kg in body mass (Table 1a). Small hunted mammals were those identified as hunted with mean weights <2kg (Table 1b). Those species identified as not commonly hunted formed the unhunted category. Only species with >30 occurrence records were retained for subsequent analysis (following Wisz *et al*., 2008); those excluded are listed in Table 1.

## Environmental parameters

Spatially explicit data were extracted using ArcMap 10.5 (ESRI, California, USA) and nine environmental parameters were generated to test their explanatory power in predicting local species presence (Figure 1b). These included distance to a) roads, b) ecotourism camps and/or research stations, c) the park boundary, and e) villages as generated by the Near function of the Spatial Analyst toolbox. Villages were located using local knowledge and their locations ground-truthed during 2017 using a Garmin GPSMAP 64s. The road network was georeferenced by driving all access tracks during 2017. Forest cover was based on data from Hansen *et al.* (2013) with the area deforested since 2000 within 2km of each species record generated using the Focal function of the Spatial Analyst toolbox based on changes in Hansen *et al.* (2013). Pixels with forest cover data in 2000 but subsequently deforested had their values changes to zero before analysis. The park zone within which each record was recorded was categorically classified as core or buffer. In addition, mean annual temperature (bio1) and total annual precipitation (bio12) were download from www.worldclim.org and downscaled to a 30m resolution from 1km through the Resample function in the Raster Processing toolbox, using a bilinear method based on elevation of a Digital Elevation Map (DEM). In many ecosystems, water availability is a constraining factor in mammalian distributions (e.g. see Lamprecht, 1990) but the high, steep elevations of Cusuco National Park mean that significant bodies of standing water do not exist (i.e. there are no large ponds or lakes). Being predominately cloud forest, the region has a high density of small streams which, along with an abundance of phytotelmata (water available from reservoirs in plant leaves and trunks) means that water is never limiting. Thus, beyond inclusion of annual precipitation, distance to water was not included in our analysis.

## Species Distribution Models

Species Distribution Models were run for each species separately using MaxEnt version 3.4.0 (Phillips *et al*., 2017). Various programs and packages provide opportunities to create SDMs; MaxEnt provides an intelligible interface (including the ability to adjust model parameters, intuitively interpretable outputs,

TABLE 1. Species inventory of indirect tracks and signs recorded during walked transect surveys throughout Cusuco National Park from 2006 to 2017.

| **Species** | **No. of records** | **Percentage of records (%)** |
|---|---|---|
| **i) Retained for analysis** | | |
| **a) Large hunted** | | |
| Tapirus bairdii (Baird's tapir) | 397 | 19.5 |
| Mazama temama (Central American deer) | 423 | 20.8 |
| Dasypus novemcintus (nine-banded armadillo) | 258 | 12.7 |
| Pecari tajacu (white collared peccary) | 31 | 1.5 |
| **Sub-total** | 1,109 | 54.4 |
| **b) Small hunted** | | |
| Cuniculus paca (lowland paca) | 258 | 12.7 |
| Dasyprocta punctata (Central American agouti) | 38 | 1.9 |
| **Sub-total** | 296 | 14.5 |
| **c) Unhunted** | | |
| Nasua narica (white-nosed coati) | 391 | 19.2 |
| Aloutta palliate (mantled howler monkey) | 66 | 3.2 |
| Didelphis virginiana (Virginia opossum) | 65 | 3.2 |
| Conepatus semistriatus (striped hog-nosed skunk) | 45 | 2.2 |
| **Sub-total** | 567 | 27.8 |
| **ii) Excluded from analysis** | | |
| Bassariscus sumichrasti (cacomistle) | 1 | 0.0 |
| Eira barbara (tayra) | 12 | 0.6 |
| Herpailurus yogouaroundi (jaguarundi) | 9 | 0.4 |
| Leopardus wiedii (margay) | 24 | 1.2 |
| Orthogeomys (pocket gopher) | 10 | 0.5 |
| Potos flavus (kinkajou) | 29 | 1.4 |
| Procyon lotor (Northern raccoon) | 15 | 0.7 |
| Sciurus sp. (squirrel) | 4 | 0.2 |
| Urocyon cinereoargenteus (gray fox) | 12 | 0.6 |
| **Sub-total** | 60 | 2.9 |
| **Total** | **2,037** | **100.0** |

projections that can be easily exported to produce multi-taxa projections and it works faster than R-based packages e.g. biomod2 (Phillips *et al*., 2017; de Marco & Nobrega 2018).

Model background points were generated along the line transect network to represent an equal number of pseudoabsences to presence records. To account for variation in survey effort per transect, additional pseudoabsences were generated (following Kramer-Schadt *et al*., 2013) such that more were generated on transects that were surveyed with greater frequency in proportion to the number of times surveyed. A combination of linear and quadratic species response curves were used to avoid model overfitting (using threshold or hinge features) and to generate ecologically plausible responses (de Marco and Nobrega, 2018). Data were subsequently split randomly into a training set (consisting of 75% of presence/pseudoabsence data) and a test set (25% of data). Models were run with up to 100-iteration replicate bootstrapping (generating a different 75:25 random split for each run) such that a measure of variation (standard deviation) could be generated when assessing model performance i.e. the Area Under the Curve (AUC) value for the Receiver Operating Characteristic (ROC) curve.

There is a general move away from using AUC as an SDM evaluation metric as it can be problematic where values are influenced by the extent of model prediction (Smith 2013). If the extent of the model is large and the species has a restricted distribution within that extent, then AUC values can be artificially inflated by assuming meaningful absence outside the species range extent (Merow *et al*., 2013). Alternative metrics of model fit (sensitivity, specificity, True Positive Rate, True Skill Statistic or Kappa) are not without their own issues (Allouche *et al*., 2006). In our case, the extent of the study area was, by SDM standards, very small (less than 23 × 15km), pseudoabsences were constrained to surveyed transects (thus more closely representing true absence than random background points generated through unsurveyed areas) and most modelled species were widespread throughout the surveyed area (i.e. at equilibrium); thus the assumptions of AUC were met and it was deemed the most suitable and straightforward evaluation metric.

The predicted probabilities for each species were aggregated *post-hoc* into four groups; i) all species, ii) large hunted species, iii) small hunted species and iv) unhunted species, defined as above. The average probability of occurrence across each of the four groups was plotted on heat maps using ArcMap. Using 1km buffers for each village, the mean probability of occurrence for each of the four species groups was determined and villages ranked in order of their likely surrounding mammalian diversity. A Spearman's correlation was used to test if mean mammal predicted probabilities were associated with the human population size of each village (extracted from the last National Census).

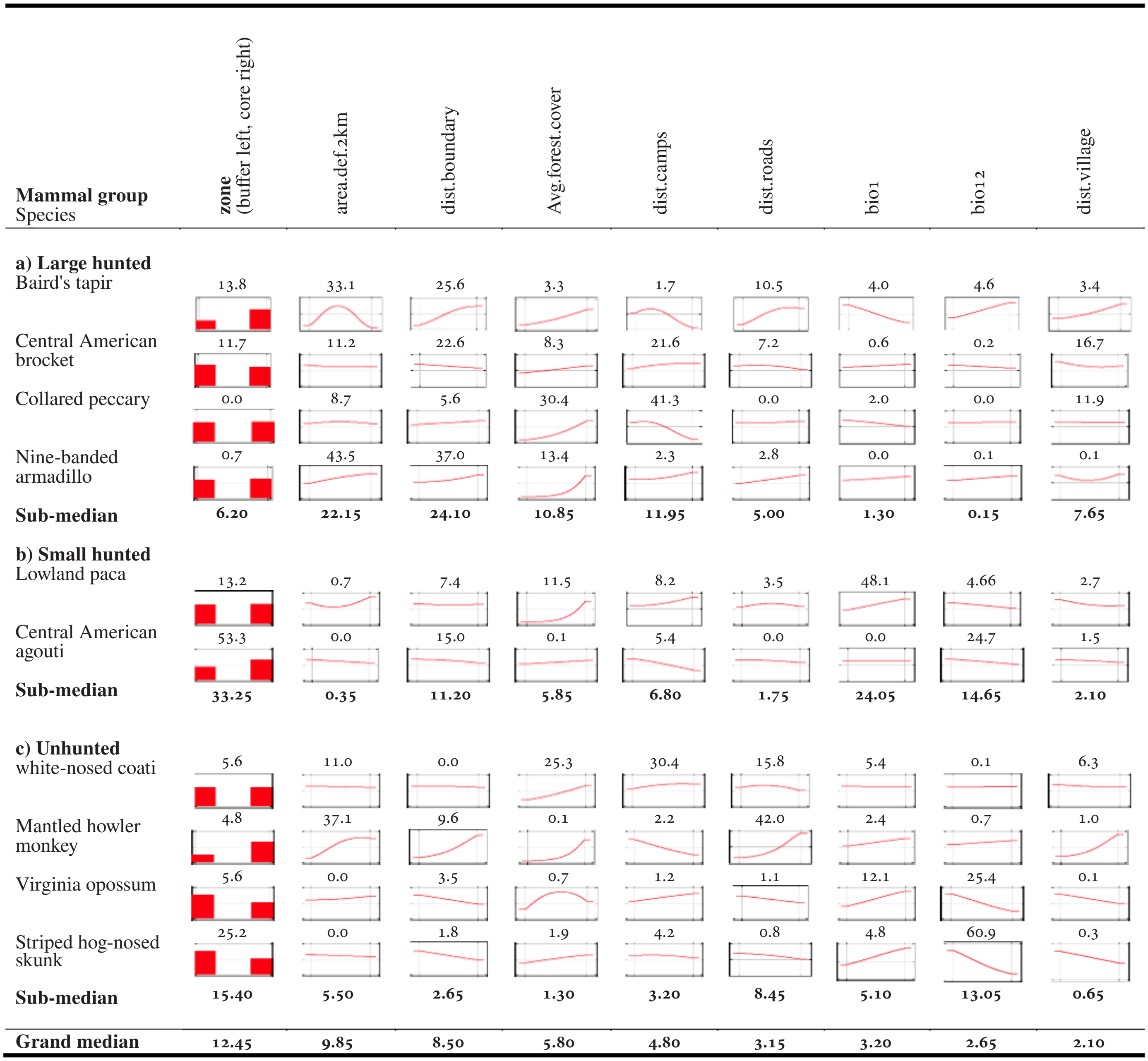
## Results

A total of 2,037 species-specific presence records were collected with notable variation in the number of records between species (Table 1).

Training set AUC values for species-specific SDMs ranged from 0.58 for Central American brocket deer to 0.83 for the mantled howler monkey (Table 2). Training and test set AUC values were comparable and highly correlated ($r_s$=0.902. $p$<0.001, $n$=10) with test set AUC values ranging from 0.56 also for Central American brocket deer (and white-nosed coati) to 0.82 for the mantled howler monkey. Mean AUC values across aggregated groups suggested that prediction success was largely comparable for large hunted, small hunted and unhunted species (Table 2).

The percentage contribution of each environmental parameter to species-specific models varied idiosyncratically, as did species-specific response curves (Figure 2). For example, zonal protection contributed 56% to the Virginia opossum SDM where it was more likely to be common in the buffer zone, 0% to the collared peccary SDM where it was equally likely to occur in the core and buffer zones and 14% to the Baird's tapir SDM where it was more likely to occur in the core zone. Three variables contributed most to species distributions when assessed across all species aggregated together: zonal protection, deforestation within 2km and distance to the park boundary (Figure 3; blue bars).

Four species were more likely frequent in the core zone (Baird's tapir, nine-banded armadillo, Central



| **Mammal group**<br>Species | **zone** (buffer left, core right) | area.def.2km | dist.boundary | Avg.forest.cover | dist.camps | dist.roads | bio1 | bio12 | dist.village |
|---|---|---|---|---|---|---|---|---|---|
| **a) Large hunted** | | | | | | | | | |
| Baird's tapir | 13.8 | 33.1 | 25.6 | 3.3 | 1.7 | 10.5 | 4.0 | 4.6 | 3.4 |
| Central American brocket | 11.7 | 11.2 | 22.6 | 8.3 | 21.6 | 7.2 | 0.6 | 0.2 | 16.7 |
| Collared peccary | 0.0 | 8.7 | 5.6 | 30.4 | 41.3 | 0.0 | 2.0 | 0.0 | 11.9 |
| Nine-banded armadillo | 0.7 | 43.5 | 37.0 | 13.4 | 2.3 | 2.8 | 0.0 | 0.1 | 0.1 |
| **Sub-median** | **6.20** | **22.15** | **24.10** | **10.85** | **11.95** | **5.00** | **1.30** | **0.15** | **7.65** |
| **b) Small hunted** | | | | | | | | | |
| Lowland paca | 13.2 | 0.7 | 7.4 | 11.5 | 8.2 | 3.5 | 48.1 | 4.66 | 2.7 |
| Central American agouti | 53.3 | 0.0 | 15.0 | 0.1 | 5.4 | 0.0 | 0.0 | 24.7 | 1.5 |
| **Sub-median** | **33.25** | **0.35** | **11.20** | **5.85** | **6.80** | **1.75** | **24.05** | **14.65** | **2.10** |
| **c) Unhunted** | | | | | | | | | |
| white-nosed coati | 5.6 | 11.0 | 0.0 | 25.3 | 30.4 | 15.8 | 5.4 | 0.1 | 6.3 |
| Mantled howler monkey | 4.8 | 37.1 | 9.6 | 0.1 | 2.2 | 42.0 | 2.4 | 0.7 | 1.0 |
| Virginia opossum | 5.6 | 0.0 | 3.5 | 0.7 | 1.2 | 1.1 | 12.1 | 25.4 | 0.1 |
| Striped hog-nosed skunk | 25.2 | 0.0 | 1.8 | 1.9 | 4.2 | 0.8 | 4.8 | 60.9 | 0.3 |
| **Sub-median** | **15.40** | **5.50** | **2.65** | **1.30** | **3.20** | **8.45** | **5.10** | **13.05** | **0.65** |
| **Grand median** | **12.45** | **9.85** | **8.50** | **5.80** | **4.80** | **3.15** | **3.20** | **2.65** | **2.10** |

**Figure 2** Species-specific response curves to environmental parameters with the relative contribution to each model shown as the numerical value above each plot. Variables are ranked (left to right) in descending order of their averaged contribution (grand median) across all species. x-axis = variation in the focal variable; y-axis = probability of species occurrence.

American agouti and the mantled howler monkey); two species were more likely frequent in the buffer zone (Virginia opossum and striped hog-nosed skunk) and four had near equal likelihood of occurrence in both zones (Central American brocket deer, collared peccaries, lowland paca and white-nosed coati). Deforestation contributed substantially to three species SDMs with the nine-banded armadillo and mantled howler monkey being positively associated with landscape-level forest loss. Baird's tapir exhibited a quadratic response to deforestation being most common at intermediate levels

TABLE 2 SDM performance as estimated by the Area Under the Curve (AUC) value of the Receiver Operating Characteristic (ROC) curve for each species from 100 iteration model runs where the data were split randomly (75:25) into a training and test set.

| **Species** | **AUCE ± SD** | |
|---|---|---|
| | **Training set** | **Test set** |
| **a) Large hunted** | | |
| *Tapirus bairdii* (Baird's tapir) | 0.79 | 0.78 ± 0.02 |
| *Mazama temama* (Central American brocket deer) | 0.58 | 0.56 ± 0.03 |
| *Dasypus novemcintus* (nine-banded armadillo) | 0.59 | 0.57 ± 0.11 |
| *Pecari tajacu* (Colared peccary) | 0.69 | 0.60 ± 0.10 |
| **Sub-mean** | 0.67 | 0.64 ± 0.05 |
| **b) Small hunted** | | |
| *Cuniculus paca* (lowland paca) | 0.62 | 0.60 ± 0.04 |
| *Dasyprocta punctata* (Central American agouti) | 0.58 | 0.59 ± 0.10 |
| **Sub-mean** | 0.65 | 0.59 ± 0.07 |
| **a) Unhunted** | | |
| *Nasua narica* (white-nosed coati) | 0.79 | 0.78 ± 0.02 |
| *Aloutta palliate* (mantled howler monkey) | 0.58 | 0.56 ± 0.03 |
| *Didelphis virginiana* (Virginia opossum) | 0.59 | 0.57 ± 0.11 |
| *Conepatus semistriatus* (striped hog-nosed skunk) | 0.69 | 0.60 ± 0.10 |
| **Sub-mean** | 0.67 | 0.64 ± 0.05 |
| **Grand mean** | **0.68 ± 0.08** | **0.78 ± 0.02** |

of forest loss. Two variables contributed most to large hunted mammal distributions: distance to park boundary and deforestation (Figure 3; red bars). Baird's tapir, collared peccaries and nine-banded armadillos likely occurred more frequently further from the park boundary (i.e. in the interior) while Central American brocket deer were likely marginally more common closer to the park boundary. Two variables contributed most to small hunted mammal distributions: zonal protection and bio1 i.e. mean annual temperature (Figure 3; green bars). Central American agouti were more likely frequent in the core zone while lowland paca were positively associated with mean annual temperature. Two variables contributed most to unhunted mammal distributions: zonal protection and bio12 i.e. annual rainfall (Figure 3; orange bars). Virginia opossum and striped hog-nosed skunk were strongly negatively associated with annual rainfall.

When pooled together, large hunted mammal ranges were contributed to more by deforestation within 2km, distance to the park boundary, forest cover, distance to ecotourism camps and/

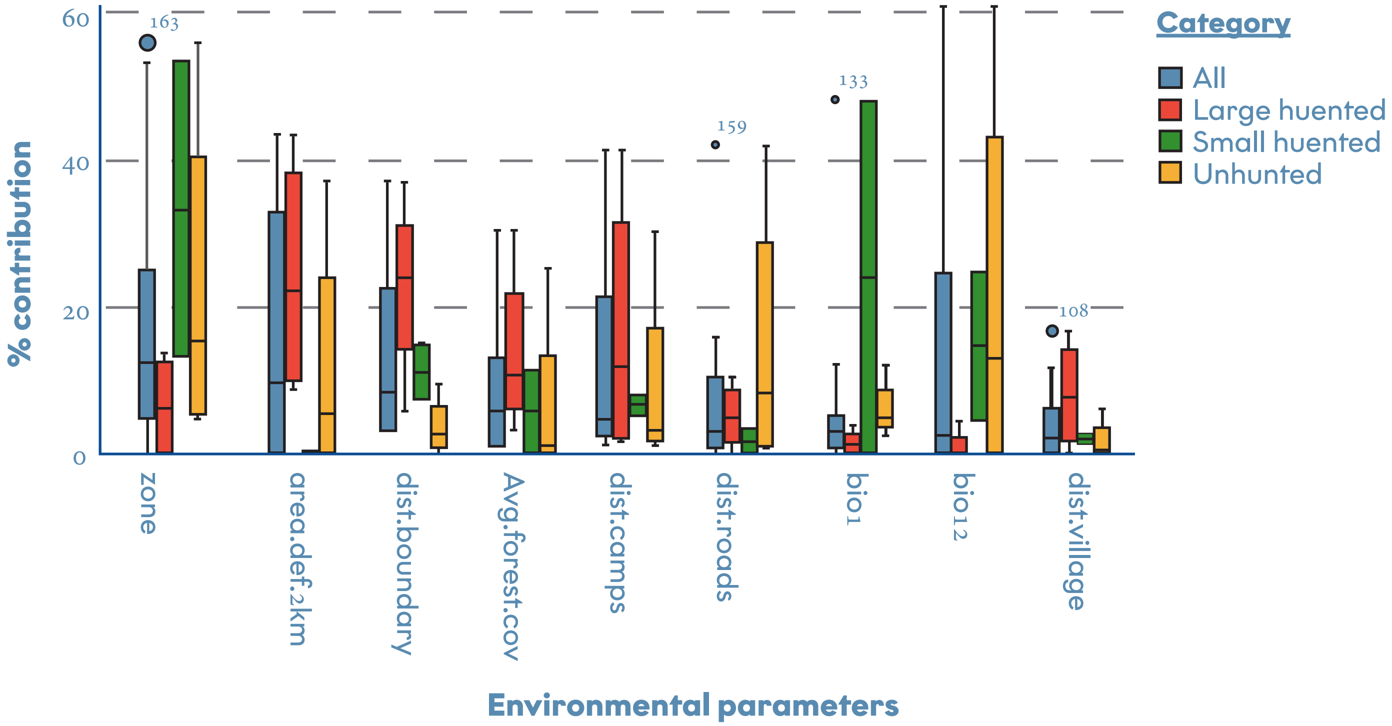


**Figure 3** Percentage contribution of predictor variables to SDMs across **(a)** all species, **(b)** large hunted, **(c)** small hunted, and **(d)** unhunted species. Boxplot shows the interquartile range (box spanning 25th-75th percentiles), median (line) and 95% confidence intervals (whiskers). Variables are ranked (left to right) in descending order of their median values.

or research station, and distance to villages than any other group (Figure 3). Zonal protection contributed more to small hunted and unhunted species than large hunted species. Distance to road contributed more to unhunted species than large or small hunted species. Mean annual temperature (bio1) and annual rainfall (bio12) contributed more to small hunted and unhunted species than large hunted species.

The idiosyncratic nature of species-specific responses to environmental parameters resulted in a range of predicted distribution patterns (Figure 4). Baird's tapir, collared peccaries, mantled howler monkeys and to a lesser extent, Central American agouti had greatest probabilities of occurrence within the interior of the park (though the driver for this was not always zonal protection but favourable collinear environmental conditions resulting from the elevational differences between the core and buffer zones). Nine-banded armadillos and Virginia opossum had greatest probabilities of occurrence in the peripheral regions of the park. Central American brocket deer and white-nosed coati were both widespread whilst environmental conditions seemed favourable for striped hog-nosed skunk only in the southeast of the park. Despite species-specific variation in the distribution of landscape favourability, when aggregated, the highest probability of occurrence for all species was in the north-west portion of the core zone and lowest in and around deforested patches in the north and west of the park buffer zone. Broadly, the same pattern was evident for large hunted mammals but the disparity in favourability between the core and buffer of the park was more pronounced. A similar pattern was observed for small hunted mammals though these were more likely to occur in the buffer zone than large hunted mammals and were least likely to occur at highest elevations (lowest mean temperatures and highest rainfall) in the western half of the core zone. Unhunted mammals had the most spatially uniform probability of occurrence throughout the park but with a lower probability of occurrence in and around deforested patches in the north and west of the buffer zone.

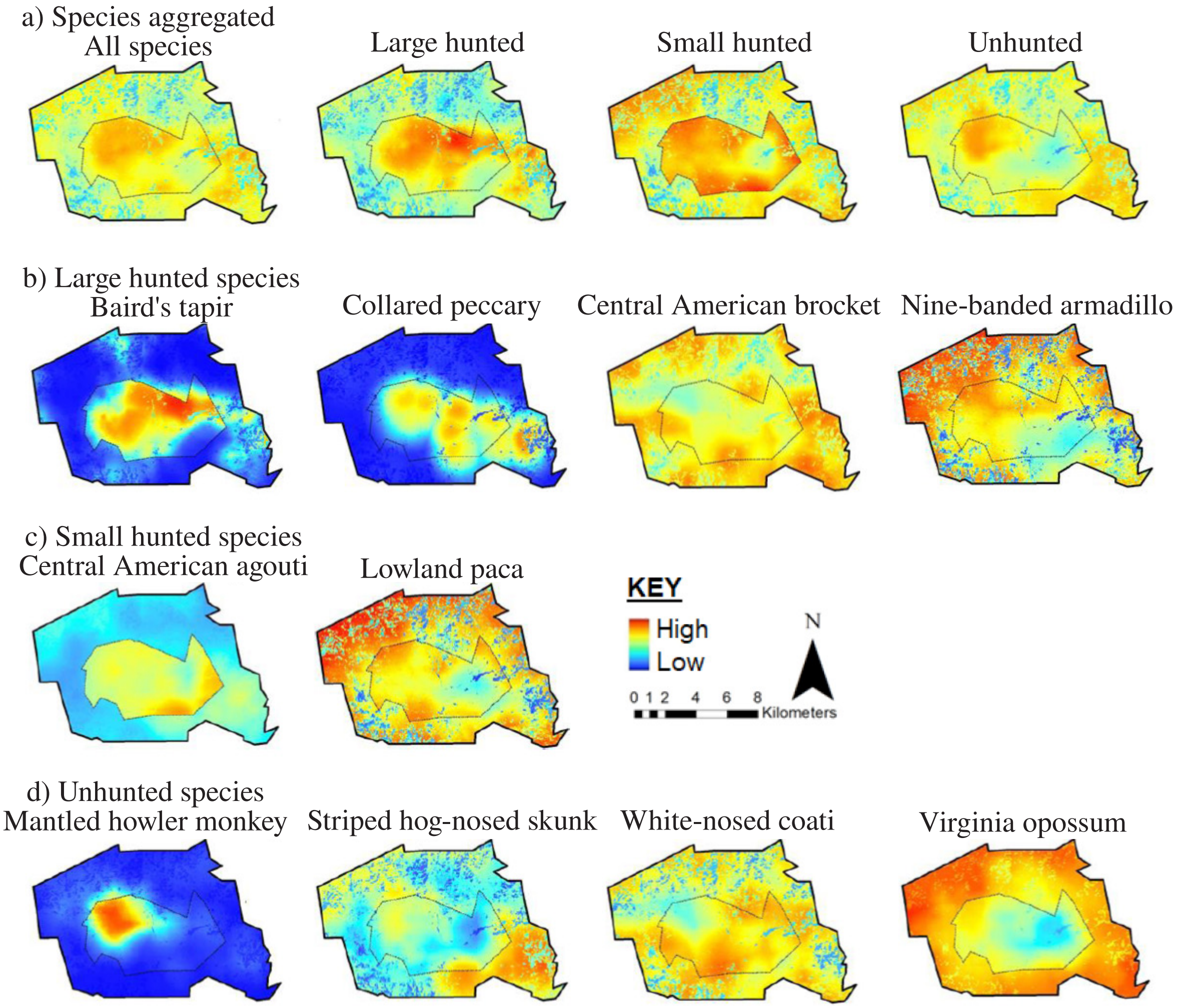


**Figure 4** Spatial variation in SDM predicted probabilities for landscape suitability for terrestrial mammals showing **(a)** species aggregated into categories (all species, large hunted, small hunted and unhunted) with species-specific model outputs for named species within each: **(b)** large hunted **(c)** small hunted and **(d)** unhunted species.

Villages differed in the average probability of mammalian species group occurrence within a 1km buffer (Table 3, Figure 5) with those located on the edge in the south of the buffer zone (Regadio) and in the north of the buffer zone associated with the lowest probability of large hunted mammal occurrence (specifically the villages of Santo Tomas and Corpus). Despite some individual species SDMs responding to Distance to Village, none of the mean predicted probabilities of each mammal group around villages were significantly correlated with village population size ($r_s$>-0.193, *p*>0.126; Table 3)

## Discussion

This study suggests that despite the highest level of protected area designation terrestrial mammal distribution (and by extension, community composition) within remnant patches of Neotropical cloud forest may be driven predominately by local anthropogenic factors. Sources of bioclimatic variation (e.g. temperature or rainfall) which ordinarily limit species range edge boundaries, do not constrain species at the local scale due to lack of sufficient spatial variation. Thus, local SDMs do not rely on defining positive relationships to determine a species optimal environmental niche but rather are more likely to be informed by negative relationships describing parochial species absence. As some species will be more tolerant of human disturbance than others

TABLE 3 Suitability of the landscape (1km buffer) surrounding each village within the buffer zone of Cusuco National Park for **(a)** all, **(b)** large hunted, **(c)** small hunted and **(d)** unhunted mammal species allowing conservation awareness and legislative protection enforcement to be targeted.

| | | | Predicted probability ± SD | | | | |
|---|---|---|---|---|---|---|---|
| # | Village name | Coordinates | Human population | a) All species | b) Large hunted | c) Small hunted | d) Unhunted |
| 1 | Colorado | 15°28′59″N, 88°11′11″W | 40 | 0.56 ± 0.06 | 0.54 ± 0.08 | 0.55 ± 0.07 | 0.59 ± 0.04 |
| 2 | Bañaderos | 15°30′46″N, 88°10′22″W | 311 | 0.52 ± 0.13 | 0.51 ± 0.16 | 0.50 ± 0.12 | 0.54 ± 0.11 |
| 3 | La Fortuna | 15°29′19″N, 88°15′27″W | 800 | 0.51 ± 0.07 | 0.49 ± 0.09 | 0.62 ± 0.12 | 0.48 ± 0.06 |
| 4 | La Estrella | 15°33′32″N, 88°20′53″W | 136 | 0.51 ± 0.08 | 0.42 ± 0.08 | 0.62 ± 0.10 | 0.55 ± 0.08 |
| 5 | Las Palmas | 15°34′56″N, 88°18′37″W | 120 | 0.50 ± 0.10 | 0.42 ± 0.10 | 0.59 ± 0.12 | 0.53 ± 0.10 |
| 6 | Nueva Eden | 15°31′34″N, 88°11′31″W | 220 | 0.49 ± 0.13 | 0.47 ± 0.15 | 0.48 ± 0.13 | 0.52 ± 0.11 |
| 7 | Santo Tomas | 15°33′42″N, 88°18′01″W | 173 | 0.48 ± 0.09 | 0.38 ± 0.09 | 0.58 ± 0.10 | 0.53 ± 0.08 |
| 8 | Buenos Aires | 15°30′02″N, 88°10′53″W | 371 | 0.47 ± 0.13 | 0.45 ± 0.16 | 0.45 ± 0.11 | 0.51 ± 0.12 |
| 9 | Tierra Santa | 15°34′29″N, 88°17′27″W | 248 | 0.46 ± 0.11 | 0.43 ± 0.18 | 0.50 ± 0.14 | 0.47 ± 0.09 |
| 10 | Nueva Esperanza | 15°32′45″N, 88°14′39″W | 320 | 0.43 ± 0.09 | 0.42 ± 0.13 | 0.41 ± 0.09 | 0.44 ± 0.06 |
| 11 | Corpus | 15°34′42″N, 88°15′40″W | 180 | 0.43 ± 0.09 | 0.37 ± 0.09 | 0.49 ± 0.12 | 0.46 ± 0.08 |
| 12 | Regadio | 15°27′59″N, 88°18′04″W | 163 | 0.42 ± 0.11 | 0.39 ± 0.11 | 0.45 ± 0.14 | 0.44 ± 0.09 |
| | Mean/Total | | 3,082 | 0.47 ± 0.10 | 0.44 ± 0.14 | 0.53 ± 0.13 | 0.48 ± 0.09 |

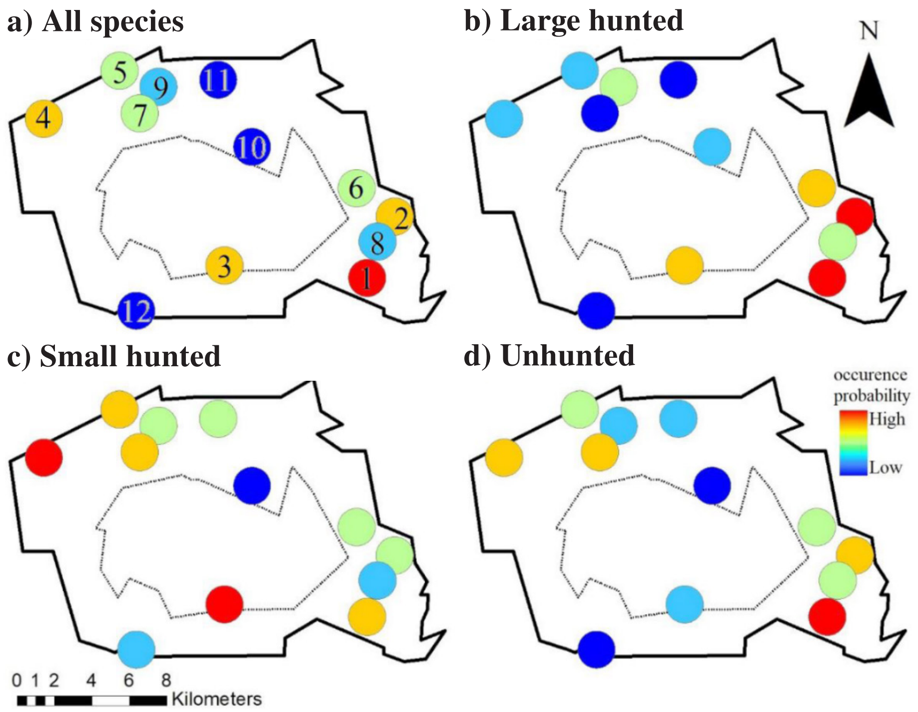


**Figure 5** Probability of occurrence for each mammals group where those villages with the highest occurrence probability are labelled red and the lowest in blue. Numbered labels correspond to Table 3.

will, the distribution of tolerant species may fail to be predicted adequately as few variables constrain their distribution at the local scale. For example, the Central American brocket deer is widespread, ranging from Mexico to Colombia, inhabiting perennial forest, cloud forest, sub-perennial forest and low-dry forest (Reyna-Hurtado and Tanner 2005, Bello *et al*., 2016) including transformed secondary forests and cropland (Naughton-Treves *et al*., 2003). It is highly adaptable and can switch habitat selection, for example, in areas with high hunting intensity brocket deer may use sub-optimal habitats (Reyna-Hurtado and Tanner, 2005). Indeed, the probability of brocket deer occurrence was relatively high throughout Cusuco National Park, indicating it is a widespread species. Consequently, despite its species-specific SDM having poor predictive power (low AUC value) we can be relatively confident that the species exhibited few strongly negative responses to local anthropogenic disturbance despite the high likelihood that it is hunted for bushmeat (perhaps suggesting a certain population robustness to harvesting). A similar pattern was observed for some other common and widespread species such as the Virginia opossum.

Anthropogenic disturbance was notably most important in determining the distribution of large hunted mammal species; specifically, deforestation within 2km, distance to the National Park boundary, distance to ecotourism camps and/or research stations (i.e. populated camps) and distance to villages, than for any other mammalian grouping. Mammals are known to respond to the proximity of villages (Schussler *et al*., 2018, Remis and Kpanou, 2011) indicative of the numerous human disturbances in-and-around settlements; most notably forest access permitting destructive practices (Oldekop *et al*., 2016) and nuisance disturbance from noise and/or smell (Francis and Barber, 2013). However, the predicted probability of mammalian groups within 1km around villages was unrelated to human population size suggesting it may be local activities (hunting, deforestation, disturbance etc.) impact mammals rather than human population *per se*. Ecotourism camps and/or research stations were camps temporarily occupied (during June-August annually) by visiting scientists, students and school children. It may be hypothesised that noise and smell associated with even temporary occupation may be sufficient to cause some species to exhibit local avoidance behaviour, moving away from camps. It may also be that temporary infrastructure (transects) could be co-opted by local hunters after the research season (September-May annually) utilised for forest access on hunting trips. However, species-specific responses suggest that the probability of the occurrence of some species was higher closer to (rather than further away) from such camps. Thus, rather than demonstrating an expected negative effect of proximity to camps, our analyses may suggest a recording bias. This may arise when surveyors who started observations at research stations working out towards the end of each radial transect may be more likely to detect tracks and signs early in surveys when attention is highest whilst signs may be missed later (i.e. further from camp) when attention is waning *aka* 'observer fatigue' (Morrison 2016). This directly contrasts with species occurrence patterns derived from camera trapping calling into question some of the utility of tracks and sign surveys (Hoskins, 2020).

Some large hunted species, for example tapir, were positively associated with distance to the National Park boundary with probability of occurrence peaking in the interior of the core zone, furthest from external threats and pressures. Deforestation contributed to the distributional patterns of three species; nine-banded armadillo and mantled howler monkey appeared positively associated with landscape-level forest loss while tapir exhibited a quadratic response (occurring most commonly at intermediate levels of forest loss). This seems unintuitive and likely represents a correlational rather than causal relationship i.e. it seems unlikely that armadillos or howler monkeys actively select landscapes vulnerable to deforestation.

Armadillos were most likely to occur in the north and west of the park, which happens to be an area of high local deforestation, however, the drivers of armadillo occurrence in this region may be independent of forest loss. In Cusuco National Park, newly deforested patches are typically in areas of pristine (90-100%) forest cover whilst overall landscape levels of forest loss are <7% i.e. even where deforestation risk is highest, relatively little forest has been lost (Hoskins, 2020). Mantled howler monkeys can tolerate human disturbance to an extent (Rimbach *et al*., 2013) including forest fragmentation (Rangel-Negrin *et al*., 2014, Michalski and Peres, 2007). Species like tapir may be associated with high canopy cover (Carrillo-Reyna *et al*., 2015) and thus their occurrence may be associated with regions of high forest cover which in Cusuco National Park is associated with deforestation risk (Hoskins, 2020) yet individual species records never occurred within deforested landscape patches (also see Rodrigues and Chiarello, 2018). Thus, positive relationships with deforestation may be collinear with forest cover. Moreover, species responses to deforestation may be time-lagged and not immediately realised (Zimbres *et al*., 2013). Hence, pooling species occurrence data from 2006 to 2017, although necessary to generate sufficient sample sizes in terms of numbers of detections, may fail to capture the temporal response to disturbance in light of ongoing and temporally variable patterns of forest loss.

From 2000 to 2017, a total of 17.6km² (>7%) of Cusuco National Park was deforested with some of the highest rates of forest loss in more recent years (Hoskins, 2020). The area is under considerable pressure likely driving species declines and defaunation (Hoskins *et al*., 2020). Tapir tracks and signs were recorded only in the core zone of the park where density was estimated at 0.39 individuals/km² between 2006 and 2011 (McCann *et al*. 2012) comparing favourably with tapir densities throughout Central America which range from 0.08 - 1.19 individuals/km² (Williams, 1984; Fragoso, 1991; Wright *et al*.,1994; Naranjo, 1995; Foerster, 1998; Carbonell & González, 2000; Tobler, 2000). However, given the small total extent of the park's core zone at just 77km², the total tapir population of Cusuco National Park was estimated at just 30 individuals in 2012 with the isolated, islandised, nature of the population suggesting imminent risk of local extirpation (McCann *et al*. 2012) which has been largely realised with no tracks and signs recorded during the last year of survey in 2018 (Hoskins *et al*., 2020). Whilst tapir may still occur in Cusuco National Park it seems the population is precariously close to local extinction.

Zonal protection contributed most to the distribution of small hunted and unhunted species. The effects of zonal protection varied across species with some found mostly in the core zone (e.g. Central American agouti), while others mostly occurred in the buffer zone (e.g. Virginia opossum). The effects of zonal protection may be partly confounded, not only by distance from the National Park boundary, but also elevation (and thus mean annual temperature and rainfall) as these are associated with the core, which is at higher altitude (up to 2,200 metres above sea level) than the rest of the park (which extends down to near sea level). Nevertheless, MaxEnt deals with multicollinearity in spatial variation of explanatory variables by adjusting species response curves to some degree, however, when variables are near analogues this adjustment is unlikely to successfully partition effects. We maintained zonal protection as a variable in models as there was east-west variation in elevation and thus temperatures and rainfall (even within the core zone). Buffer zones are used in protected area management to create greater distance between human disturbance and the core protected area. They allow for some restricted use of resources (e.g. managed hunting and/or timber extraction) to divert exploitation from core zones (see Lynagh and Urich, 2002). The importance of buffer zones surrounding protected areas has been demonstrated in the Neotropics (Paolino *et al.*, 2016) but much like the planning of core protected areas, arbitrary delineation may be counterproductive (van der Meer *et al.*, 2014, Lima and Ranieri, 2018, Geneletti and van Duren, 2008). Whilst some species (for example, tapir) had lowest probability of occurrence in Cusuco National Park's buffer zone, others were positively associated with this region (e.g. Virginia opossum). This is indicative of a robustness to disturbance (smaller bodied mammals may be less vulnerable to landscape level impacts), a lack of being targeted by hunting (opossum are distasteful) or robustness to hunting (high fecundity and short generation times).

Distance to roads contributed to the ranges of unhunted species more than large or small hunted species. Virginia opossum, white-nosed coati and striped hog-nosed skunks had a negative association with distance to roads (i.e. were most likely to occur close to access tracks). These species are omnivorous and are positively associated with humans generally (Markovchick-Nicholls *et al.*, 2008, Daily *et al.*, 2003). Low sample size must also be considered here and thus generalisations made with caution.

Virtually all species occurrence were positively correlated with forest cover. Higher cover suggests more intact forest which is likely to have greater resources (i.e. niche space) than lower cover forest whilst mammals themselves may create a positive feedback loop where forest cover is higher where a diverse mammal community exists due to their role in seed dispersal due to frugivory and thus forest (re)generation (Nagy-Reis *et al.*, 2017).

Mammal surveys were conducted consistently during June and July annually providing a regular snapshot of mammalian activity, nevertheless, it should be noted that this study is pertinent to the spatial distribution of mammals during the dry season only. This seasonal bias could have influenced the spatial patterns of mammalian distributions as species may alter their movements and habitat selection synchronised with, for example, rain-induced plant flowering, fruiting and the associated mass emergence of insects which drive reproduction in some species (Medellín & Redford, 1992). Moreover, temporal trends in local climatic conditions (which were drier in more recent years associated with climate change) may have influenced temporal patterns in mammal distributions driving shifts in species distributions associated with longer-term changes in, for example, plant phenology. Climate change is known to disproportionately impact cool/wet-adapted montane species driving many to higher altitudes overtime (Neate-Clegg *et al.*, 2018); the so-called 'escalator to extinction'. Thus, the spatial patterns in mammalian distributions described here are likely to change in future.

The applied conservation utility of this study lies in ranking villages within Cusuco National Park by the predicted probability of mammalian occurrence in the surrounding landscape i.e. we identified villages by name which had lower large mammal occurrence indicative of greater human impacts. This information will facilitate Government departments, forestry managers, conservation organisations, and patrolling wardens to target their protection efforts (including legislative enforcement and prosecution) to those villages that have greatest negative impact (for example, Santo Tomas and Corpus in the north-west of the park buffer zone among others such as Regadio on the southern park boundary).

# Conclusions

Local SDMs may appear at first a poor method in determining the factors constraining species distributions as they fail to capture global range-wide constraints such as extreme thresholds for tolerating temperatures or rainfall (El-Gabbas and Dormann, 2018) but here we demonstrate their utility in assessing local anthropogenic drivers of Neotropical cloud forest mammal distributions. Disturbance is more likely to define species ranges at the local scale and we show that even with the highest level of protected area designation i.e. National Park status, the ranges of Neotropical mammals are nevertheless driven predominately by anthropogenic factors. Our approach facilitates spatial targeting of conservation investment to specific named local areas most vulnerable to anthropogenic impacts. It is hoped that this should increase targeted conservation, education and reduce disturbance to the remaining mammal community of Cusuco National Park.

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## Data availability statement

The data used in this study (mammal species locational records and shapefile rasters for environmental variation) are archived within the Supporting Information in the file *Cusuco_mammal_records*_2006-2017.*xlsx* and zipped folder *Cusuco_environmental_layers*.

## Supporting information

*Cusuco_mammal_records*_2006-2017.*xlsx*
*Cusuco_environmental_layers* zipped folder

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# Pioneering a fungal inventory at Cusuco National Park, Honduras

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## Abstract

Neotropical cloud forests are biologically and ecologically unique and represent a largely untapped reservoir of species new to science, particularly for understudied groups like those within the Kingdom Fungi. We conducted a three-week fungal survey within Cusuco National Park, Honduras and made 116 collections of fungi in forest habitats at 1287–2050 m a.s.l. Undescribed species are likely to be present in those collections, including members of the genera *Calostoma* (Boletales), *Chlorociboria*, *Chlorosplenium*, *Ionomidotis* (Helotiales), *Amparoina*, *Cyathus*, *Gymnopus*, *Pterula* (Agaricales), *Lactifluus* (Russulales), *Mycocitrus* (Hypocreales), *Trechispora* (Trechisporales), and *Xylaria* (Xylariales). In this paper, we discuss the contributions and impacts of mycological surveys in the Neotropics and propose the establishment of a long-term mycological inventory at Cusuco National Park—the first of its kind in northern Central America.

## Resumen extendido

**Antecedentes:** Los bosques nublados neotropicales son únicos tanto biológica como ecológicamente, y representan un reservorio de especies no exploradas por la ciencia, particularmente para grupos poco estudiados como el reino de hongos. Dentro de la cadena alimentaria, los hongos juegan un papel clave en el flujo de energía a través de los ecosistemas y proporcionan nutrición para muchos organismos. A pesar de esto, los estudios a largo plazo de las comunidades fúngicas son escasos, por lo que se crean brechas en nuestra comprensión de la diversidad de hongos, así como las interacciones interespecíficas e intraespecíficas en las que están involucradas. Aquí, presentamos las contribuciones y los estudios micológicos en el neotrópico y proponemos el establecimiento de un inventario micológico a largo plazo en el Parque Nacional Cusuco, el primero de su tipo en el norte de América Central.

**Metódos:** Durante tres semanas del verano de 2019, recolectamos hongos en el Parque Nacional Cusuco (1287–2050 m s. n. m.), un área protegida de bosque nublado en la cordillera Merendón, noroeste de Honduras. Nos hemos enfocado en tres tipos de colecciones: macrohongos productoras de cuerpos fructíferos (colectados en áreas de 10 × 10m, en cada punto de muestro dentro de los transectos) y levaduras aisladas del filoplano de diferentes plantas (aisladas de hojas recolectadas en los transectos) y los microhongos del orden Laboulbeniales en moscas ectoparásitas de murciélagos (colectados durante los muestreos de murciélagos en redes de niebla). Todas las muestras fueron enviadas a la Universidad de Purdue para su futura identificación.

**Resultados:** Los resultados preliminares de este muestreo son un total de 116 colectas (sin incluir levaduras del filoplano o Laboulbeniales), de los cuales 91 se han identificado a nivel de orden, 65 a nivel de género y 25 a nivel de especie. De estos, 34 colecciones fueron identificadas como *Agaricales*, seguidos por once colecciones de Helotiales y Hypocreales. Se identificaron un total de 37 géneros; los mejor representados fueron *Marasmius* (10 colecciones), *Xylaria* (7) y *Amanita* (4). Hasta la fecha, generamos y analizamos secuencias de la región ITS para un total de 17 colecciones. Al realizar un BLAST en NCBI GenBank, diez secuencias compartieron entre 89.05 y un máximo de 97.60% de identidad con secuencias conocidas. Estos resultados indican que esas colecciones representan especies no descritas o especies conocidas no secuenciadas para esta región de ADN. Los géneros con especies potencialmente no descritas son: *Calostoma* (Boletales), *Chlorociboria, Chlorosplenium, Ionomidotis* (Helotiales), *Amparoina*, *Cyathus, Gymnopus, Pterula* (Agaricales), *Lactifluus* (Russulales), *Mycocitrus* (Hypocreales), *Trechispora* (Trechisporales) y *Xylaria* (Xylariales).

**Discusión:** Dada la escasez de colecciones de hongos de bosques nubosos, la presencia de organismos microendémicos en el Parque Nacional Cusuco y los resultados preliminares descritos aquí, es probable que numerosas nuevas especies de hongos estén esperando ser descubiertas en esta área. Por este motivo, se iniciará un proyecto de largo plazo de muestreo de hongos en el Parque Nacional Cusuco con el objetivo de describir la diversidad de hongos (macrohongos, hongos del filoplano y aquellos asociados con artrópodos) con base en la información morfológica, ecológica y molecular. Las alarmantes tasas de destrucción del hábitat son una de las principales amenazas del Parque Nacional Cusuco, por lo que existe una necesidad urgente de recolectar y documentar estas especies de hongos, especialmente las potencialmente endémicas —antes de que desaparezcan—. El estudio a largo plazo de los hongos del parque permitirá examinar las tendencias temporales en las comunidades fúngicas en relación con la variabilidad climatológica y la creciente perturbación ambiental. Así como los proyectos multidisciplinarios de Operation Wallacea también permitirán determinar qué tan bien se correlacionan las tendencias demográficas en las comunidades fúngicas con otros taxones, otra vía de investigación que en este momento sigue siendo poco explorada a escala global.

# Introduction

The Kingdom Fungi includes a highly diverse group of organisms, in terms of both species richness as well as the ecological roles they deliver. Their unique biological characteristics such as polarized hyphal growth, external enzymatic digestion of macromolecular nutrients, and the production of large amounts of reproductive mito- and meiospores have made the fungi one of the evolutionarily most successful groups on the planet, colonizing almost every available habitat and substratum (Cannon *et al.* 2018; Sheldrake 2020). Fungi fulfil essential roles in many ecosystems, constituting three major ecological strategies (Piepenbring *et al.* 2016; Willis 2018): 1) saprotrophs, or 'recyclers' of the natural environment, decay dead organic material, releasing its carbon and nutrient content back into the ecosystem; 2) mutualists, such as mycobionts in lichens, mycorrhizal fungi, and ruminant gut fungi derive their nutrients from other living organisms while providing benefits to their host, and 3) parasites and pathogens, or 'regulators' of the natural environment, obtain their nutrients from other living organisms and are essential in controlling host populations and maintaining biodiversity (Connell 1971).

Fungi are an important component of the food chain in terrestrial ecosystems, being a factor in the flux of energy through ecosystems and providing nutrition for many different animals—vertebrates (e.g., bonobos, tortoises, potoroos) as well as invertebrates (collembolans, mites, nematodes) (Fogel & Trappe 1978; Claridge *et al.* 1996, 1997; Verbeken 2002). For at least the past 5,000 years (Peintner *et al.* 1998), mushrooms have been used by humans for their nutritive and medicinal properties (Dong *et al.* 2015; Du *et al.* 2015; Zhang *et al.* 2015; Jumbam *et al.* 2019). Species in the genera *Agaricus*, *Ganoderma*, *Hericium*, and *Lentinula* are among the most popular ones, and are both collected by professional and amateur mycologists and domesticated and cultivated in mushroom farms around the world. Some of the most prized mushroom in genera such as *Boletus*, *Cantharellus*, *Morchella*, and *Tuber* —this last containing the most expensive food items in the world (Luard 2006; Patel *et al.* 2017)— form ectomycorrhizal associations and are hard to grow in axenic culture. We depend on fungi and the metabolites they produce in the pharmaceutical world (e.g., penicillin, cyclosporine) and in the food industry (e.g., alcohol, citric acid). On the other hand, fungi are the cause of devastating infectious diseases (e.g., *Batrachochytrium dendrobatidis* in amphibians, *Cryphonectria parasitica* in chestnut trees, *Pseudogymnoascus destructans* in bats, *Puccinia graminis* in wheat; Leonard & Szabo 2005; Warnecke *et al.* 2012; Rigling & Prospero 2018; Scheele *et al.* 2019; Fisher & Garner 2020), of food spoilage (Pitt & Hocking 2009), and of human infections, particularly in immunocompromised patients (Sharma *et al.* 2014; Ioannou *et al.* 2019).

Apart from Arthropoda, fungi constitute the most diverse group of eukaryotes on the planet. Since the start of modern fungal taxonomy with Micheli's (1729) *Nova Plantarum Genera*, 148,000 species have been formally described (Hibbett *et al.* 2016; Cheek *et al.* 2020). The number of described species has increased from 1,000–1,500 per year about 10 years ago to 2,073 species in 2019 (Cheek *et al.* 2020). These numbers may seem startling but are only a fraction of the estimated number of species, which ranges from a conservative 1.5 million (Hawksworth 1991) to 6.0 million species (Taylor *et al.* 2006) or more. While we are getting close to knowing all species in groups such as the flowering plants and birds, the gap between numbers of described and estimated species is huge for fungi and insects, where the bulk of species is still undescribed as of today (Mora *et al.* 2011; Hawksworth 2017). Alliances like the International Barcode of Life (http://ibol.org/; Adamowicz 2005) try to fill this gap, e.g., with multi-million-dollar funded programs aimed at generating DNA barcodes for hundreds of thousands of species (Hobern & Hebert 2019). The fact that the knowledge of fungi lags in comparison to most other groups of organisms can be explained by a number of factors: many fungi are microorganisms occupying ecological niches in very specialized substrates or habitats. Another important reason is that many areas, which are often hotspots for fungal diversity, remain underexplored. And it is here that tropical mycology comes in the picture. In tropical and subtropical areas, the diversity of biotic and abiotic factors, including high density and diversity of potential host species, high humidity, and the availability of a plethora of microhabitats, might be expected to promote high fungal diversity.

Distributions of macroorganisms typically follow a latitudinal gradient wherein their diversity is negatively linked to increasing latitude (Hillebrand 2004; Mannion *et al.* 2014). In contrast, distributions of microorganisms, such as the fungi, which produce propagules that are microscopic in size, are often perceived as cosmopolitan (De Wit & Bouvier 2006), although lack of data from tropical regions has not allowed for rigorous testing of this hypothesis. A recent study based on environmental DNA from hundreds of soil samples from around the world found that diversity of both saprotrophic and parasitic soil fungi was highest in the tropics; signatures of endemism were also highest in the tropics (Tedersoo *et al.* 2014). In other words, the latitudinal gradient model is a better fit for patterns of fungal diversity. One exception noted by the authors concerns the guild of ectomycorrhizal fungi —those that are mutual associates with specific woody trees— where diversity increased towards temperate and boreal ecosystems. These results, however, may be more reflective of depauperate sampling of ectomycorrhizal fungi in tropical systems than of true trends; for example, where ectomycorrhizal communities have been studied in depth in tropical systems, species richness equals or exceeds that in most temperate systems (Smith *et al.* 2011).

To date, most fungi have been described by European and North American mycologists. This bias is reflected in type localities of described species. For example, 85% of genera of Leotiomycetes are based on a species described from temperate Europe and to a lesser extent North America (Johnston *et al.* 2019; MyCoPortal 2020); only 5% are based on species described from tropical areas, the eastern Palearctic, or the temperate Southern Hemisphere, with the fewest descriptions from Africa. The total number of type collections from the African continent is 15 in a class of over 6,000 species. As a further indicator of this temperate ecosystem bias, from 2,189 fungal species described in 2017, only 12% originated from the Neotropics and only 4% from Africa (Niskanen *et al.* 2018), whereas Europe and Asia accounted for 25% and 35%, respectively. This clearly illustrates the major gap in our knowledge of tropical and subtropical fungi (Aime & Brearley 2012), while knowledge of tropical diversity is absolutely necessary in order to gain a complete picture of phylogeny and evolutionary history. E.J.H. Corner, botanist and mycologist at the Singapore Botanical Gardens, commented in 1946 on botanists' ignorance of the true variety of tropical forests and later stated that "mycology would be nothing without the tropics."

The exploration of tropical biodiversity and including tropical sampling in our datasets has often drastically changed our perspective on evolutionary relationships. This can be illustrated with the inclusion of mainly tropical African and Asian species in phylogenetic analyses of Russulaceae, one of the dominant ectomycorrhizal groups in all ecosystems worldwide. Whereas the largest genus, *Russula*, is hypothesized to have a Northern Hemisphere origin (Buyck *et al.* 2020), *Lactifluus* (one of the milkcap genera) is a primarily tropical genus likely of African origin, from where it radiated to different continents (De Crop 2016; De Crop *et al.* 2017). Understanding the evolution of Russulaceae in function of climate and available host trees, is only possible if tropical representatives are included. As stated before, many taxa in tropical areas (at the level of species, but also at higher taxonomic levels) are endemic and represent key factors in our evolutionary reconstructions. The exploration of ectomycorrhizal diversity in the Neotropics is in urgent need. Many groups (such as *Lactifluus*) are now relatively well-studied in tropical Africa and southeastern Asia, but it is only since the turn of the 21st century that neotropical hotspots such as the Guiana Shield and the Brazilian Amazon have received more attention.

Humans have been classifying organisms since antiquity (Lennox 1980), with the Linnaean taxonomic system having been in place for over 250 years (Linnaeus 1758). Nevertheless, the coverage of many taxonomic groups by this system is nowhere near complete (Brito 2010)—including the fungi, particularly tropical ones. There is no such thing as an inventory of tropical fungi. In fact, only a few historic initiatives were undertaken documenting neotropical fungal diversity. These generally covered either specific groups of fungi or limited geographic ranges (e.g., Guevara & Dirzo 1998; Pérez & Camino 2000; Henkel *et al.* 2002; Chaverri & Vílchez 2006; Baroni *et al.* 2009; Piepenbring *et al.* 2011). A proposal for an All-Taxa Biodiversity Inventory (ATBI) of fungi at the Area de Conservación Guanacaste in Costa Rica, sparked by a ten-day workshop, demonstrates the enormous effort necessary just to compile a complete inventory of recorded species (Rossman *et al.* 1998). The authors reported a total of 1,200 known fungal taxa in Costa Rica (data from BPI).

One of the limitations of fieldwork is seasonal fruiting phenology of most macrofungi. Although macrofungi are present year-round as visibly undetectable microorganisms within the substrate, they are only visible, e.g., as mushrooms, during a brief fruiting period that is induced by genetic and environmental factors, such as rain. As an example, a 21-year study in a single plot in a temperate forest found that the above-ground fungal diversity was very different from what is "hidden away" in the substrate (soil, dead wood, inside living tissue, etc.) (Straatsma *et al.* 2001). Of a total of 408 recorded species in this study, only 2% were annually collected, and 5% were found for the first in year 21. During fieldwork, we are limited to those groups of fungi that are forming visible ascomata and basidiomata (Buyck *et al.* 2010; De Crop *et al.* 2019; Jumbam *et al.* 2019). When understudied regions of the world, such as many remote sites in the Neotropics, are being sampled for the first time, no herbarium collections or data are available to plan on a timeline for maximizing the probability of collecting fruiting bodies.

Some of the most extensive fungal documentation in Central America in the last decade has occurred in Panama. Piepenbring (2007) compiled an annotated checklist of 1,807 fungal species in Panama based on literature data and concluded that the unveiling of fungal diversity in the country is in the pioneering phase. A large effort for long-term monitoring of fungal diversity in the country was subsequently initiated, with monthly sampling along a 500-m transect in secondary vegetation in lowland of western Panama. Piepenbring *et al.* (2012) reported 567 species of fungi and fungus-like organisms, of which two were undescribed and 19 represented new country reports. None of the simulated species estimators approached an asymptote. Intriguingly, the Whittaker plot showed a steep curve with a long "right tail" accounting for 50% singletons and 18% duplicates (meaning, rarely recorded species), a hallmark for hyperdiverse and undersampled habitats (Piepenbring *et al.* 2012). We expect this to be true for most neotropical areas and argue that long-term fungal surveys are needed to document fungal species diversity and distributions of species, which may be affected by human-caused habitat destruction and global warming, as well as to fill gaps in our understanding of how fungal taxa are related to one another.

In 2019, an opportunistic fungal survey was conducted by the first author in Cusuco National Park, a 23,440-ha protected area in the Merendón range, northwestern Honduras, under the auspices of Operation Wallacea. In this paper, we present preliminary results from this inventory and lay out a roadmap towards an enduring standardized fungal inventory in this area as part of Operation Wallacea's multi-taxa biodiversity monitoring and surveillance programme in Honduras (Martin *et al.* 2021).

## Fungal survey in Cusuco National Park, 2019

### Methods

Opportunistic surveying of fungi took place in Cusuco National Park (hereafter abbreviated as CNP, located at 15°32′31″N 88°15′49″W) between 22 June and 13 July 2019. See Martin *et al.* (2021) elsewhere in this Special Issue for a summary of habitats and environmental conditions in this area. Surveys were limited to transects and survey plots around Base Camp (1572 m a.s.l.) and Guanales (1287 m a.s.l.); one Cantiles transect was sampled as well (Mochilero Trail, *c.* 2050 m a.s.l.). During this survey, we focused on three types of field collections: macrofungal fruiting bodies, yeasts isolated from leaf surfaces (= phylloplane yeasts), and bat fly-associated microfungi. Above-ground, ephemeral ascomata and basidiomata of non-lichenized fungi were collected along transects and at 10 × 10m² survey plots. Fresh specimens were photographed *in situ* and at the camp. Specimens were assigned a HONDURAS19-F collection number and their metadata were recorded, including data, specific locality, geographic coordinates (when available), substratum, and surrounding habitat notes. Tentative names were assigned to specimens based on initial morphological examination. Samples were placed in plastic containers or paper bags and transported back to camp. Rice-sized pieces of tissue or 1–6 cups/peridioles were removed from each fresh specimen and stored in 1.5 ml Eppendorf tubes prefilled with either 600 µl of Nuclei Lysis Solution (Wizard Genomic DNA Purification Kit, Promega, Madison, WI) or 400 µl of AP1 buffer + 4 µl of RNase A (100 mg/ml) (DNeasy Plant Mini Kit, Qiagen, Valencia, CA). Specimens were dried with silica gel at the camp site.

We also obtained fungal strains from various leaf surfaces collected along transects to generate data on phylloplane fungal microorganisms (Toome *et al.* 2013; Haelewaters *et al.* 2020b). Leaves were cut into 1 × 1cm² pieces that were attached with Vaseline Petroleum Jelly to the inner lid of a Petri dish containing agar media. Cultures were maintained on potato dextrose agar (PDA, Difco, Livonia, Michigan) supplemented with chloramphenicol (1 mL L21) to limit bacterial growth. Petri dishes were monitored daily for growth of colonies, which were transferred with sterilized toothpicks in 2 ml screw-cap microcentrifuge tubes prefilled with PDA medium supplemented with chloramphenicol. After shipment to the M.C. Aime laboratory, pure cultures were streaked out and stored at 4 °C. Finally, we collected bat flies during nightly bat surveys (Medina-van Berkum *et al.* 2021). Neotropical bat flies have been reported as hosts for Laboulbeniales fungi in the genera *Gloeandromyces* and *Nycteromyces* (Haelewaters *et al.* 2021).

All collections and tubes with tissue samples were sent to PUL (Kriebel Herbarium, Purdue University, West Lafayette, USA). Specimens will be deposited at GENT, PUL, and TEFH (*sensu* Thiers continuously updated). At the molecular lab at Purdue University, DNA extractions were performed with the DNeasy Plant Mini Kit and Wizard Genomic DNA Purification Kit following the manufacturers' instructions. PCR amplification targeted the internal transcribed spacer (ITS) of the ribosomal RNA gene (rDNA), the region proposed as the fungal DNA barcode for identification (Schoch *et al.* 2012), using primers ITS1f and ITS4 (White *et al.* 1990; Gardes & Bruns 1993). PCR conditions followed Haelewaters *et al.* (2020b).

## Preliminary results

Mycological survey work at CNP has only recently been initiated and all data were preliminary at the time of writing. A total of 116 collections were made in 2019 (not including phylloplane-inhabiting microfungi or Laboulbeniales), of which 91 have been identified to order-level, 65 to genus, and 25 to species. Of these, 34 collections were identified as Agaricales, the typical mushroom-formers, followed by 11 collections of both Helotiales and Hypocreales (Table 1). A total of 37 genera were identified; best represented were *Marasmius* (10 collections), *Xylaria* (7), and *Amanita* (4). To date, we generated and analyzed ITS sequences for a total of 17 collections, submitted to NCBI GenBank under accession numbers MT571521–MT571537. When blasting in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi), ten of our newly generated ITS sequences shared between 89.05 and maximum 97.60% identity with known sequences. Using an identity threshold of 98.5% (see discussion in Lücking *et al.* 2020), these results imply that those collections represent either undescribed species or species that are currently unsequenced for this barcode. The genera with potentially undescribed species are: *Calostoma* (Boletales), *Chlorociboria*, *Chlorosplenium*, *Ionomidotis* (Helotiales), *Cyathus*, *Gymnopus*, *Mycena*, *Pterula* (Agaricales), *Mycocitrus* (Hypocreales), *Trechispora* (Trechisporales), and *Xylaria* (Xylariales) (Table 2).

An interesting case is HONDURAS-F017, our single collection of a *Lactifluus*. Upon initial blast, our ITS sequence for HONDURAS-F017 was 100% identical to *Lactifluus deceptivus* (KR364101, De Crop *et al.* 2016). However, recent phylogenetic analysis revealed that this taxon is a complex of multiple species (Delgat *et al.* 2019); blasting against the ex-lectotype sequence of *L. deceptivus* (GenBank accession number MN251093) only resulted in a 96.47% match. In their recent paper, Delgat *et al.* (2019) described several new species in the *L. deceptivus* complex. Among them is *L. domingensis*, with which the sequence of our Honduran collection shares 99.16% identity (Table 2). A simple ITS tree (based on RAxML, Stamatakis 2014) showed that the Honduran collection in fact represents an undescribed sister species of *L. domingensis*.

# Discussion

Hawksworth & Lücking (2017) stipulated that "about 85,000 of the currently accepted fungal species have no sequence data available." In other words, for 57% of described species, no DNA sequences have been generated (Cheek *et al.* 2020). It is possible that potentially new taxa based on barcode divergence may be representative of those species for which no sequence data exist. On the other hand, a number of studies have highlighted high degrees of fungal endemism in tropical cloud forest ecosystems, such as the one sampled in this study (Lodge *et al.* 2008; Smith *et al.* 2013; Bandala *et al.* 2016). Del Olmo-Ruiz *et al.* (2017) evaluated fungal diversity and patterns of endemism in Neotropical montane cloud forests. The authors found that there is little overlap in species of fungi reported from Mesoamerica (*sensu* Myers *et al.* 2000), the Caribbean, and South America and that fungal species richness was highest in Mesoamerican cloud forests. The same authors also built a Mexican dataset and identified three regions of endemicity supported by parsimony and Bayesian approaches. As a result, Del Olmo-Rui *et al.* (2017) suggested that thousands of undescribed species inhabit tropical montane cloud forests, some with very restricted distributions and potentially in danger of disappearing. Likewise, limited sampling on the remote highest peak of the Maya Mountains in Belize, known as Doyle's Delight, a lower montane subtropical cloud forest, has led to the discovery of numerous new fungal species and genera (e.g., Baroni *et al.* 2007, 2008, 2009; Ortiz-Santana *et al.* 2007; Lodge *et al.* 2008; Ginns *et al.* 2010; Lindner *et al.* 2011; Ovrebo *et al.* 2011; Niveiro *et al.* 2020). With regard to our sequence data, given the reported high levels of fungal endemism from these habitats, we predict that many undescribed species are to be discovered at CNP (Table 2).

Starting in 2022, a more formal fungal ATBI project will be initiated at CNP. The goals of this monitoring initiative are to describe the diversity of fungi encountered in the park based on morphological, ecological, and molecular information; and to assess potential changes in fungal abundance and fungal species diversity over multiple years. This formal survey work will consist of systematic searches on Operation Wallacea's existing network of transects and habitat plots in CNP (details in Martin *et al.* 2021). Two transects per camp will be surveyed, with four randomly selected 10×10m survey plots that are located on each transect. On each survey plot two random subplots of 2×2m will be chosen, with 30 minutes of intensive sampling per subplot being conducted. Each subplot sampling event will be followed by processing of collections, which includes taking notes, sorting specimens, taking tissue for DNA, et cetera. Subplots will be located adjacent to the existing multi-taxa composite habitat plots along the transects, which are also being monitored for environmental variables, to characterize the soil, epiphyte density, number of samplings, and overall vegetation density. In this way, our survey methods will allow collection of not just distribution data, but comparative data on fungal diversity variation among habitats, which environmental variables define these variations, how fungal diversity changes over time, and how patterns in fungal species richness correlate with those in other surveyed taxa.

In addition to the systematic surveys targeting typical non-lichenized ascomata and basidiomata in the field, we will also collect microfungi from undersampled guilds, especially phylloplane fungi and those associated with Arthropoda. The latter includes representatives of Hypocreales and Laboulbeniales, which both remain poorly understood in the tropics (Araújo & Hughes 2016; Blackwell *et al.* 2020). For these arthropod-inhabiting fungi, mycologists will work closely with entomologists as well as mammalogists (to collect ectoparasitic flies from bats)—highlighting the inter-disciplinary collaborative approaches that we are undertaking, as per Haelewaters *et al.* (2021).

TABLE 1. Overview of macrofungal collections identified to order-, genus-, and species-level; number of genera and species identified, and number of undescribed species expected in Cusuco National Park based on the 2019 fungal survey.

| Order | No. collections | Genera | | Species | | |
|---|---|---|---|---|---|---|
| | | Collections | Identified | Collections | Identified | Undescribed |
| Agaricales | 34 | 26 | 13 | 5 | 5 | 4 |
| Auriculariales | 1 | 1 | 1 | 1 | 1 | 0 |
| Boletales | 7 | 4 | 3 | 3 | 2 | 1 |
| Geastrales | 1 | 1 | 1 | 0 | 0 | 0 |
| Helotiales | 11 | 5 | 4 | 3 | 3 | 3 |
| Hymenochaetales | 2 | 2 | 2 | 2 | 2 | 0 |
| Hypocreales | 11 | 4 | 2 | 3 | 2 | 2 |
| Orbiliales | 3 | 3 | 1 | 0 | 0 | 0 |
| Pezizales | 4 | 3 | 2 | 0 | 0 | 0 |
| Phallales | 1 | 1 | 1 | 1 | 1 | 0 |
| Polyporales | 5 | 4 | 3 | 2 | 2 | 0 |
| Russulales | 3 | 3 | 2 | 1 | 1 | 1 |
| Trechisporales | 1 | 1 | 1 | 1 | 1 | 1 |
| Xylariales | 7 | 7 | 1 | 3 | 2 | 1 |
| Totals | 91 | 65 | 37 | 25 | 22 | 13 |

Long-term fungal inventory projects, such as the one here proposed, are vital for both ecological and evolutionary studies, but almost entirely lacking in the Neotropics. A pioneering 20-year survey within the Pakaraima Mountains of western Guyana, part of the lowland Guiana Shield region of South America, has documented 1,500+ species of Agaricales of which *c.* 75% are new to science (Aime *et al.* 2004), including new genera and higher lineages (Toome *et al.* 2013; Smith *et al.* 2015; Henkel *et al.* 2016, Sánchez-García *et al.* 2016; Koch *et al.* 2017); and revealed a thriving ectotrophic system estimated to carry at least 250 species of ectomycorrhizal fungi associating with a single host genus within three 1,000 $m^2$ plots of tropical lowland forest (Smith *et al.* 2011, 2017; Henkel *et al.* 2012). This is in stark contrast to the 408 total Agaricales, of which 265 were ectomycorrhizal, within the similar 21-year study in five 300 $m^2$ plots of temperate biotrophic forest (Straatsma *et al.* 2001).

As mentioned before, the inclusion of tropical taxa in our evolutionary trees has changed the way we think about taxonomic concepts, especially at the generic and family levels. For example, based on collections from the *Dicymbe*-dominated forests in Guyana, the first and only pleurotoid Catherellaceae (Henkel *et al.* 2006), craterelloid and resupinate species of *Clavulina* (Thacker & Henkel 2004; Uehling *et al.* 2012), as well as the only smooth-spored species of Ganodermataceae (*Amauroderma coltricioides*) (Aime *et al.* 2003) were discovered. An extreme example, *Pseudotulostoma volvatum* is an ectomycorrhizal ascomycete that forms a stalked fruitbody with an exposed apical gleba and woody volvate base, reminiscent of *Tulostoma*, a basidiomycete (Miller *et al.* 2001; Henkel *et al.* 2006). A total of ten genera and more than 100 species have been described from the Guiana Shield to date but hundreds more are awaiting description (e.g., Aime *et al.* 2003, 2007, 2010; Matheny *et al.* 2003; Wilson *et al.* 2012; Henkel *et al.* 2014; Husbands *et al.* 2018). In other words, documenting these tropical oddities, only available through challenging fieldwork in remote areas, allows mycologists to question characters that have been traditionally used to delimit taxa and to reveal biases resulting from temperate-based concepts. Two undescribed species in the crust-like genus *Trechispora* from Guyana are quite interesting because they represent the first members of more than fifty in the genus (He *et al.* 2019; Species Fungorum 2020) to be stipitate rather than resupinate (Fig, 1f), again broadening the taxonomic concept of a genus that has been primarily based on temperate collections (but see Larsson 1992, 1995; Ordynets *et al.* 2015). We also sampled a previously undescribed species of *Trechispora* at CNP (Figs. 1d, 1e; Table 2) that forms resupinate basidiomata with a variable hymenophore, with young irregular poroid areas and mature regions that are irpicoid to hydnoid (Haelewaters *et al.* 2020a).

## Conclusion

*Where are the missing fungi?* Hawksworth & Lücking (2017) identified three major sources for discovery of undescribed fungi – understudied habitats, cryptic species "hidden" in well-established names, and biodiversity hotspots. Neotropical cloud forests represent a rich source of undiscovered taxa in all three of these areas. The Mesoamerican biodiversity hotspot (*sensu* Myers *et al.* 2000) holds many cloud forest ecosystems, with each cloud forest 'patch' supporting its own unique community compositions. Cusuco National Park is a cloud forest site in Honduras that has been well studied under the umbrella of Operation Wallacea, although the fungal community has remained neglected until the initiation of this project. Given the presence of micro-endemic species within other taxa at CNP (Martin *et al.* 2021), along with the preliminary results described here, it is likely that numerous new fungal species await discovery. It may be the case that any site-restricted fungi at CNP are highly threatened with extinction given the alarming rates of habitat destruction in the Park (Hansen *et al.* 2013, 2020; Hoskins 2019). Thus, there is an urgent need to collect and document these species before they disappear. The data generated from the project will be used in educational and scientific products, informing on conservation strategies (Rossman *et al.* 1998); a priority that extends to other Mesoamerican cloud forest ecosystems that are often under heavy environmental pressures (Cayuela *et al.* 2006; Magrin *et al.* 2014).

TABLE 2. Collections from Cusuco National Park identified to species level. When available, BLAST results are presented of ITS sequences generated during this study. Shown are the top BLAST result along with the percentage identity and GenBank accession number, and reference in which the reported sequence was first published. New = new species to be described after morphological study and multi-locus sequencing.

| Collection | ITS | Genus | Species | Top result | % identity | Acc. No. | Reference | New |
|---|---|---|---|---|---|---|---|---|
| Honduras19-F001 | MT571521 | *Gymnopus* | sp. 1 | *Gymnopus androsaceus* | 96.95% | MH857175 | Vu *et al.* (2019) | X |
| Honduras19-F011 | MT571522 | *Amparoina* | sp. 1 | *Amparoina heteracantha* | 94.28% | MK309785 | Na & Bau (2019) | X |
| Honduras19-F016 | MT571523 | *Trechispora* | sp. 1 | *Trechispora sp. Brazil* | 95.25% | MH290763 | K.-H. Larsson, unpublished | X[a] |
| Honduras19-F017 | MT571524 | *Lactifluus* | sp. 1 | *Lactifluus domingensis* | 99.16% | KR364101 | Delgat *et al.* (2019) | X |
| Honduras19-F022 | N/A | *Xylaria* | cf. *polymorpha* | | | | | |
| Honduras19-F024 | N/A | *Ophiocordyceps* | sp. 1 | | | | | X[b] |
| Honduras19-F027 | MT571525 | *Ionomidotis* | sp. 1 | *Ionomidotis fulvotingens* | 89.05% | KY462808 | H.-O. Baral & G. Marson, unpublished | X |
| Honduras19-F030 | MT571526 | *Panus* | *conchatus* | *Panus conchatus* | 100% | MH016880 | B.S. Kaminsky *et al.*, unpublished | |
| Honduras19-F031 | MT571527 | *Calostoma* | sp. 1 | *Calostoma sp. MDO-2018b* | 97.48% | MF521438 | M. Deloya-Olvera *et al.*, unpublished | X |
| Honduras19-F032 | MT571528 | *Chlorosplenium* | sp. 1 | *Chlorosplenium chlora* | 90.37% | MG553993 | Haelewaters et al. (2018) | X |
| Honduras19-F036 | MT571529 | *Gyroporus* | aff. *subalbellus* | *Gyroporus subalbellus* | 98.61% | EU718108 | Wilson *et al.* (2011) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Honduras19-F040 | N/A | *Phallus* | *indusiatus* | | | | | |
| Honduras19-F047 | MT571530 | *Mycocitrus* | sp. 1 | *Mycocitrus aurantium* | 97.60% | MG022158 | Loguercio Leite *et al.* (2018) | X |
| Honduras19-F055 | N/A | *Mycocitrus* | sp. 1 | | | | | X[c] |
| Honduras19-F057 | N/A | *Trichaptum* | *biforme* | | | | | |
| Honduras19-F063 | MT571531 | *Xylodon* | *flaviporus* | *Xylodon flaviporus* | 99.68% | KJ140637 | Brazee *et al.* (2014) | |
| Honduras19-F069 | N/A | *Calostoma* | sp. 1 | | | | | X |
| Honduras19-F070 | MT571532 | *Chlorociboria* | sp. 1 | *Chlorociboria aeruginosa* | 92.74% | HQ604856 | M.L. Berbee *et al.*, unpublished | X |
| Honduras19-F072 | MT571533 | *Cyathus* | sp. 1 | *Cyathus stercoreus* | 96.61% | EU784193 | Brock et al. (2009) | X |
| Honduras19-F077 | MT571534 | *Oudemansiella* | cf. *canarii* | *Oudemansiella canarii* | 99.07% | GQ892790 | Petersen & Hughes (2010) | |
| Honduras19-F082 | N/A | *Xylaria* | cf. *polymorpha* | | | | | |
| Honduras19-F086 | MT571535 | *Pterula* | sp. 1 | *Pterula gracilis* | 93.72% | MH861903 | Vu *et al.* (2019) | X |
| Honduras19-F095 | MT571536 | *Xylaria* | sp. 1 | *Xylaria amphithele* | 94.78% | GU300083 | Hsieh *et al.* (2010) | X |
| Honduras19-F098 | N/A | *Auricularia* | *auricula-judae* | | | | | |
| Honduras19-F099 | MT571537 | *Favolus* | cf. *tenuiculus* | *Favolus tenuiculus* | 98.83% | MH211695 | B.S. Kaminsky *et al.*, unpublished | |

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[a] This species was formally described during the review process of this manuscript, as *Trechispora hondurensis* (Haelewaters *et al.* 2020a).

[b] We have not yet processed the ITS sequence for this collection. However, according to D.P. Hughes (pers. comm.) this species of *Ophiocordyceps* is undescribed. It was found on a weevil (Coleoptera, Curculionidae) at the Mochilero Trail of Cantiles.

[c] This represents the same species as collection Honduras19-H047. Both collections were found in close proximity of each other and are morphologically identical.

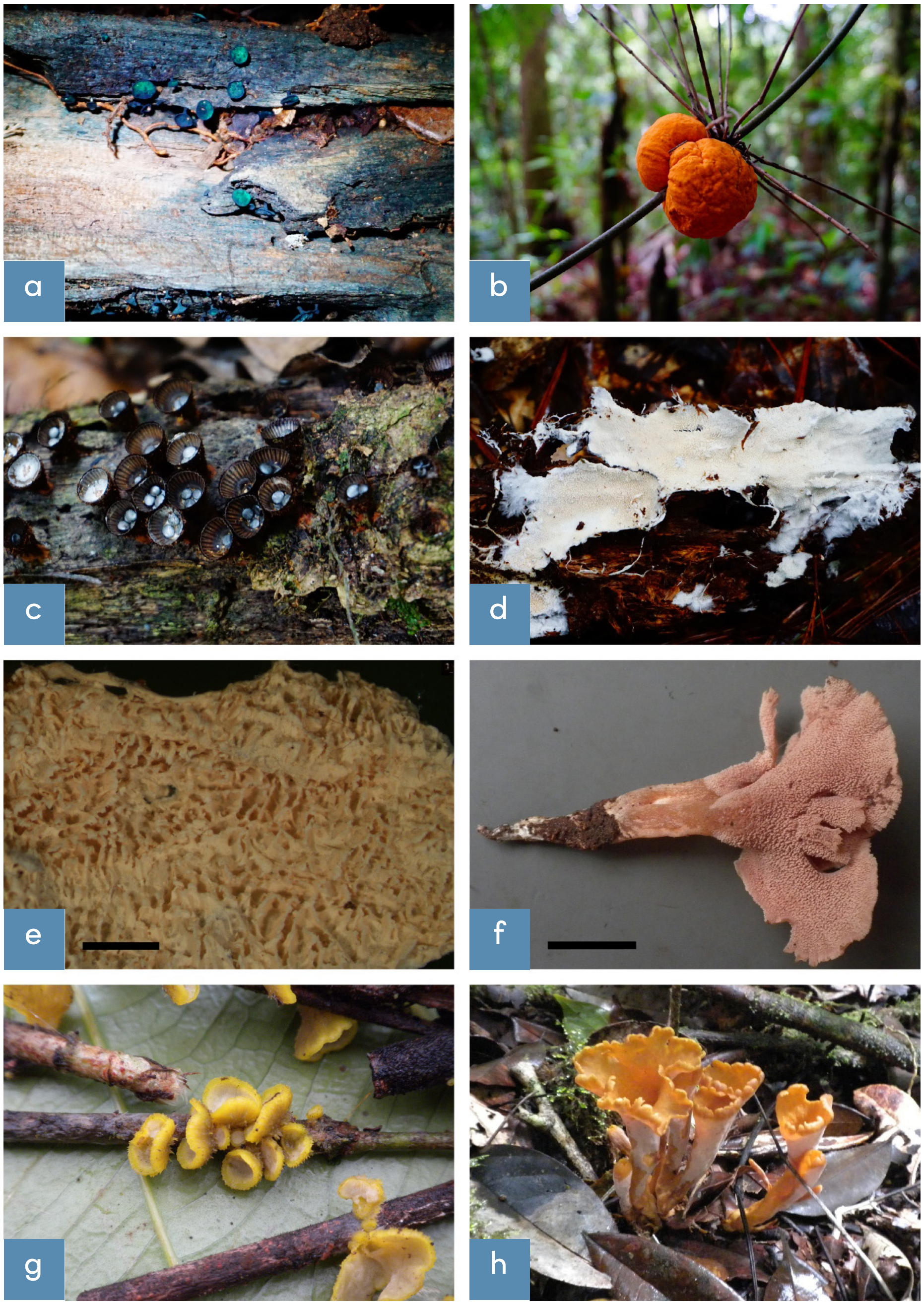

**Figure 1.** A variety of neotropical fungi, collected in Cusuco National Park, Honduras (**a–e**) and the Guiana Shield, Guyana (**f–h**). **a.** *Chlorociboria* sp. 1, collected at night during a mist-netting survey for bats. **b.** *Mycocitrus* sp. 1 growing on the nodes of bamboo. Unknowingly what it was in the field, we dubbed this fungus "bamboo brain". **c.** *Cyathus* sp. 1, a bird's nest fungus. **d–e.** *Trechispora hondurensis* [as *Trechispora* sp. 1 in Table 2], a crust-like fungus. **e.** Detail of hymenophore showing an irpicoid to hydnoid area, atypical for this genus. **f.** A stipitate, undescribed species of *Trechispora*. Thus far, no stipitate representatives have been described in the genus. **g.** *Cantharellus pleurotoides,* singular in the genus for its pleurotoid basidiomata. **h.** *Clavulina craterelloides,* which could be easily mistaken for *Craterellus* based on macromorphology. Scale bars: **e** = 2 mm, **f** = 1 cm.

In addition to improving taxonomic knowledge, research in CNP will also provide novel insights into macroecological patterns, shedding further light on, for example, latitudinal diversity gradients in the Kingdom Fungi. The long-term nature of this study will allow for the examination of temporal trends in fungal communities in relation to climatological variability and increasing environmental disturbance; themes that remain poorly studied throughout the tropics. The multi-disciplinary nature of the Operation Wallacea project will also allow the determination of how well demographic trends in fungal communities correlate with those in more easily-studied taxa — another avenue of research that at this point remains poorly explored on a global scale. Finally, the initiation of this project in CNP will have the benefits of educating several hundred students from all over the world each year on the ecological importance of fungi and the methods used to survey them in the field—an opportunity that is rarely available during tropical field courses.

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Submit the entire manuscript, including figures and tables, as a single Microsoft Word document (*.doc or .*docx). **Do NOT submit papers as pdf files**.

You can submit your paper via email. Contact the *Mesoamerican Biology Journal* Office at mesobiology.biovs@unah.edu.hn if you have any questions or need assistance.

## Manuscript format

1. Use 8.5” x 11” page size (letter size) with a 1” margin on all sides. Align left and do not justify the right margin. Number all pages starting with the title page and include continuous line numbers.
2. Double space throughout the manuscript, including tables, figures and tittle legends, abstract, and literature cited
3. Use Times New Roman 12-point font throughout except in figures, for which Arial is preferred.
4. Use the abbreviations provided in Section D (below) throughout the text.
5. Assemble manuscripts in this order:
   a. Title
   b. Authors
   c. Affiliations
   d. Abstract (s)
   e. Key words
   f. Text
   g. Acknowledgments
   h. Data availability statement
   i. Literature cited
   j. Tables
   k. Figure legends
   l. Figures
   m. Supplementary Information

## A. Title, authors and affiliation

**Title**
Where species names are given in the title, it should be clear to general readers what type(s) of organism(s) are being referred to, either by using Family appellation or common name when using species include original descriptor:

‘Invasion of Riverine systems by the Invasive armored catfish *Pterygoplicthys pardalis* (Castelnau, 1855)’ ***OR*** ‘Invasion of Riverine systems by *Pterygoplicthys pardalis* Loricaridae, (Castelnau, 1855)’
Titles that include a **geographic locality** should make sure that this is clear to the general reader:
‘Effect of habitat fragmentation on large mammal distribution, Merendon, Honduras’, NOT
‘Effect of habitat fragmentation on large mammal distribution in Merendon’.

**Authors**: Below title, include the author(s) name(s), affiliation(s), and unabbreviated complete address(es). Use superscript number(s) following author(s) name(s) to indicate current location(s) if different than above. In multi-authored papers, additional footnote superscripts may be used to indicate the corresponding author and e-mail address. Although geographical place names should use the English spelling in the text (e.g., San Pedro Sula, Antigua, San José), authors may use their preferred spelling when listing their affiliation (e.g., Zürich, Firenze, Brazil).

*Corresponding author.* Clearly indicate who will handle correspondence at all stages of review and publication, also post-publication. This includes answering any future queries about Methodology and Materials. Make sure that the e-mail address is given and that contact details are kept up to date by the corresponding author

# B. Abstract page

1. **Abstract:** Abstracts have maximum of 250 words for papers and reviews and 50 words for Notes. There is no abstract for Commentary papers.
2. The Abstract should include brief summaries of the objectives, materials and methods, results, and significance of findings. Do not use abbreviations or citations in the abstract.
3. ***Authors must provide a, "Resumen extendido", a comprehensive summary of the article in Spanish***. This 1–2 page expanded abstract will include the following: one paragraph summary of background/justification of the study, 1–2 paragraph summary of the methods and materials, 1–2 paragraph summary of the results, and 1–2 paragraph summary of the discussion and implications of the results."

4. Optionally, authors may elect to provide a ***Visual Abstract:*** a figure developed from the paper that is meant to stand-alone as a representation of the key findings.

# C. Key words

1. **Key words:** Provide 6-10 key words after the abstract, separated by a semi-colon (;). Key words should be in English (with the exception of taxonomic information) and listed alphabetically.

2. Include the location of the study as a key word if it is not already mentioned in the title (see example below). Key words should *not* repeat words used in the title. Avoid words that are too broad or too specific. (e.g., *Key words*: Zamiaceae; *Zamia zandovalii*; propagation; Honduras; tropical wet forest).

# D. Manuscript preparation

*DOUBLE BLIND PEER REVIEW*

This journal follows a double-blind reviewing procedure. Authors are therefore requested to submit two versions of their manuscript:

a. The full version including all author names and affiliations.
b. A blinded manuscript without any author names and affiliations in the text or on the title page. Self-identifying citations and references in the article text should either be avoided or left blank.

*TEXT FORMATTING*

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions)

1. Headings: please use no more than 3 levels of displayed headings

    a. Leave one blank like between main heading and text

2. When using previously published data in analyses please cite both the data archive(s) and the original manuscript(s) for which they were collected in the text: “We used previously archived data (Bruna et al 2011a,b) in our simulations.”, where a is the data archive and b is the publication. Be sure both citations are included in the literature cited (see below for an example):
3. Do not use footnotes in the main text.

*ARTICLE STRUCTURE*

1. *Introduction* State the objectives of the work and provide background, avoid a detailed literature survey or a summary of the results. Prior work based on the same project and/or data must be referenced, a final paragraph stating the unique contribution of the new submission must be explicitly described.
2. *Methods or Methodology* Include study area description if applicable. Clearly describe procedure used to obtain results, avoid using “following method used in”. Avoid including results,
3. *Results* Results should be clear and concise.
4. *Discussion* This should explore the significance of the results of the work, not repeat them. No extensive citations and discussion of published literature should be included.
5. *Conclusions* The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

# E. Abbreviations

abbreviations should be defined first and then used consistently throughout the paper

# F. Acknowledgments

acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full

# G. Data availability statement

A Data Availability Statement follows the Acknowledgements and must have the following format.

**Data Availability:** The data used in this study are archived at the Dryad Digital Repository (http://dx-.doi.org/10.5061/dryad.h6t7g) and Genbank (accession numbers FJ644654.1- FJ644660.1).

Authors waiting for article acceptance to archive data can insert the DOI or Accession Numbers when submitting the final accepted version. However, the article will not be published until the data availability statement is complete.

# H. Literature cited

please ensure that every reference cited in the text is also present in the reference list (and vice versa) The author is responsible for the accuracy of the references. References should be numbered consecutively in the order in which they are first cited. References given in tables or figure legends must be numbered in sequence with those in the text. References should be listed in numerical order in the reference section of the manuscript. Only works referred to in the text and already accepted for publication can be included:

Articles from journals: Names(s) and initials of all author(s): full article title. Journal name as abbreviated in Index Medicus, Volume number: first and last page numbers, year of publication.

Bozzetti, M., & Schulz, U. H. (2004). An index of biotic integrity based on fish assemblages for subtropical streams in southern Brazil. *Hydrobiologia*, *529*(1), 133–144. https://doi.org/10.1007/s10750-004-5738-6

Articles from electronic publications: Names(s) and initials of all author(s): full article title. Journal name as abbreviated in Index Medicus, DOI number, and publication date.

Canada Centre for Inland Waters, & Chemical Institute of Canada. Analytical Chemistry Division. (1975). Water quality parameters: a symposium. Retrieved from https://books.google.co.th/books?id=ohBSAAAAMAAJ

Books: Name(s) and initials of all author(s): full book title, edition, place of publication: publisher, year of publication

Bussing, W. (1998). *Peces de las Aguas Continentales de Costa Rica*. (E. de la U. de C. Rica, Ed.) (2nd ed.).

**We strongly recommend using reference management software such as Mendeley, EndNote, CITAVI or similar to simplify building the literature cited and to minimize mistakes.**

- Avoid citation of manuscripts as 'in prep.' or 'submitted'– they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication. Articles or book chapters cited as 'In press' must be accepted for publication; please include the journal or publisher.
- List works by the same author chronologically, beginning with the earliest date of publication.
- Use journal name abbreviations, which can be looked up here: http://www.ncbi.nlm.nih.gov/nlmcatalog/journals. If in doubt use the full journal name.
- Article in books, use: AZY, B. 1982. Title of book chapter. *In* G. Yaz (Ed.). Book title, pp. 24–36. Springer Verlag, New York, New York.
- For theses and dissertations use: 'PhD Dissertation' and 'MSc Dissertation'.

# I. Tables

- Please submit tables as editable text and not as images
- All tables are to be numbered using Arabic numerals. (e.g., Table 1, Table 2, etc.).
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

# J. Figures

- All figures and photographs are referred to as 'Figures' in the text
- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in the text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article/chapter and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic supplementary Material) should, however, be numbered separately.

*Figure Captions*

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Label multiple plots/images within one figure as A, B, C etc., and please ensure the panels of each plot include these labels and are referred to in the legend (e.g., FIGURE 1. Fitness of Medusagyne oppositifolia as indicated by (A) seed

set and (B) seed viability', making sure to include the labels in the relevant plot.)
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

*Figure Placement and Size*

Figures should also be submitted separately from the text, if possible.
Maps of field sites are generally included in the Supplementary Information unless they also present the results of analyses.
Final figures will be reduced. To ensure all text will be legible when reduced to the appropriate size use large legends and font sizes. We recommend using Arial for labels within figures without bolding text.

## K. Supporting information

We ask authors to place maps of field sites and figures and tables that do not have central relevance to the manuscript as online Supporting Information (SI). The SI can also be used for species lists, detailed technical methods, photographs, mathematical equations and models, or additional references from which data for figures or tables have been derived (*e.g.*, in a review paper). All such material must be cited in the text of the printed manuscript.

## L. Copyright transfer

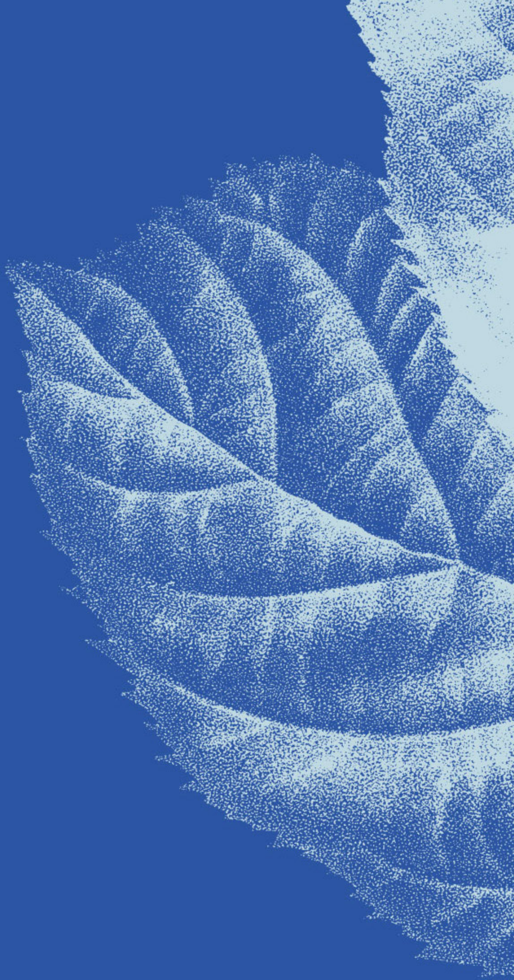
Authors will be asked to transfer copyright of the article to the Publisher (or grant the Publisher exclusive publication and dissemination rights). This will ensure the widest possible protection and dissemination of information under copyright laws.

## M. Proof reading

The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.

## N. English language editing

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood.



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