Protecting the unknown: Towards assessing extinction risk in megadiverse groups

Sérgio da Silva Henriques

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I, Sergio da Silva Henriques, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, this has been indicated.

Dedicated

to those I lost, whom I miss;

to those I gained, whom I treasure;

to those who were always with me, whom I love;

to my daughter for making me complete.

Abstract

The ongoing biodiversity crisis is one of today's most challenging and pressing global issues. Under fears that humans are currently causing the 6th mass extinction of Earth’s history, with global consequences at all levels of society, international policy targets have been set to halt biodiversity loss. This includes the United Nations’ Sustainable Development Goals and the Convention on Biological Diversity’s Aichi Targets. In order to measure progress towards these targets, several biodiversity indicators have been proposed, one of the most relevant of which is the Red List Index (RLI). In this thesis, I revise current methods to detect and monitor the extinction risk of megadiverse groups via the sampled approach to the RLI (sRLI). In the first chapter, I introduce the biodiversity crisis, current policy targets set to stop it, and the biodiversity indicators that inform them while reviewing the constraints of the currently available IUCN Red List data, focusing on the limitations of a non-random sample. In chapter 2, I revise a random sampled approach to the RLI in light of recent biodiversity targets, and using a wider dataset than originally available, I demonstrate that much smaller sample sizes than previously proposed can be representative of a group's overall trend. In chapter 3, I investigate how samples currently set to detect RLI trends can also represent a group’s overall percentage of threatened species, which I propose could be used as a new biodiversity indicator. In chapter 4, I implemented the sRLI to assess the extinction risk of a megadiverse case study group, the poorly known spiders (order Araneae), where I found data deficiency (DD) to be one of the main obstacles to the implementation of the sRLI. In chapter 5, I tackle taxonomy as one of the main reasons for DD in megadiverse groups. I propose several paths to reduce potential bias caused by taxonomy under a sRLI approach and taxonomically revise several species of a case study group, the *Eresus* and closely related *Loureedia* genus. In chapter 6, I synthesise my findings and discuss potential solutions to overcome the main issues found when implementing this protocol.

Impact Statement

This thesis showcases interconnected analyses, each with a distinct potential to impact current approaches to measure extinction risk in understudied groups, particularly those with a large number of species.

Chapter 1 raises awareness of the limitations of the data currently available in The IUCN Red List of Threatened Species, and as a non-random sample of global biodiversity, it has at times been misperceived and misused in the scientific literature. Making data limitations clear should improve future analysis of IUCN Red List data, drive conservationists to assess the extinction risk of a wider taxonomic range of species, and drive the use of random sampled approaches to IUCN Red Listing.

In chapter 2, the analysis of novel data under current policy targets and the refinement of the sampled approach to the Red List Index (sRLI) allowed us to demonstrate how much smaller samples (200 sp.) that can be more readily implemented than those currently proposed (900 sp.), can be representative of conservation trend direction. This provides an opportunity to accelerate the continuous use of the Red List Index (RLI) in comprehensively assessed groups, like mammals (last assessed over a decade ago), and to encourage the analysis of a wider range of taxa. As one of the most used biodiversity indicators to inform global policy today, such as the Sustainable Development Goals or the Aichi Targets, accelerating the use of the RLI would positively impact the accuracy of this indicator and the policies that it informs.

In chapter 3, I recognise that under the current biodiversity crisis, time is of the essence. I show that the baseline created to detect RLI trends can also be informative of a group’s overall extinction risk by estimating the percentage of threatened species. This metric has already been used informally in a policy setting (e.g. IPBES) and, if used as a biodiversity indicator, would encourage the extinction risk assessment of further taxonomic groups. Therefore, increasing the scope of the sRLI by proving that it can also be indicative of the percentage of threatened species would further impact the volume of species IUCN Red List assessments and increase available information towards meeting current policy targets.

In chapter 4, I implement the lessons from chapters 2 and 3 to a poorly studied megadiverse group, spiders. I found data deficiency (DD) to be the main obstacle to assessing the extinction risk of this group, and I analyse how this knowledge gap could be pragmatically addressed. Because this is an issue I expect other researchers to face when implementing this protocol, I propose a shift in how DD has been addressed under the sRLI framework, which would impact how other researchers approach these issues and overcome them.

The impacts of chapter 5 are mostly methodological, where I tackle one of the main causes of DD, taxonomy. I discuss how taxonomy impacts all stages of the sRLI and propose ways to incorporate new taxonomic knowledge by increasing transparency and moving towards standardisation. My results would reduce potential bias caused by taxonomically driven replacements and impact the accuracy of cross-taxa comparison.

Impact of Covid

COVID-19 has caused devastating loss of human life across the world, destroyed countless livelihoods, took a huge toll on the physical health of many and is set to cause lifelong psychological damage to many more.

This thesis was largely written during this global pandemic, under social distancing and multiple restrictions, ultimately a small price to pay for the safety of us all. During this time, I was also responsible for my young child and the restrictions imposed often made my work difficult. I have done my best to prevent the impact of the disruption caused by COVID-19 on this thesis. Despite the disruption it caused and the compromises that had to be made, I hope its completion might encourage others in a similar position.

Publications Arising From This Thesis

First authorship

The sampled approach to the red list index (Chapter 2)

Henriques, S., Böhm, M., Collen B., Luedtke J., Hoffmann M., Hilton-Taylor C., Cardoso P., Butchart S., Freeman R. (2020). Accelerating the monitoring of global biodiversity: Revisiting the sampled approach to generating Red List Indices. Conservation Letters, e12703.

The first author was responsible for reverse engineering and replicating the results of the original research that first presented the sampled Red list Index approach; engaging with relevant data holders to gather relevant updated global dataset as well as curate it in relevant ways; co-designing the experimental design to expand on the approach (under the guidance of the supervisors, and via extensive discussions with co-authors); co-write the code (with supervisors support), test and run all simulations used in the results; write the first draft (including all graphics), incorporate co-authors suggestions and submit the manuscript for publication, including accepting and enacting the suggested minor revisions by the editor and reviewers.

Taxonomy (Chapter 5)

Henriques, S., Miñano, J., Zarcos, L. P., Rezac, M., Rodríguez, F., Tamajón, R., & Avilés, J. M. (2018). First records of Loureedia (Araneae, Eresidae) from Europe, with the description of a new species and a survey of the genus. Revista ibérica de aracnología, (33), 3-20.

The first author compiled, reviewed, synthesised and rectified all relevant literature about this family; engaged with a global network of experts that had observed or worked on similar species (many of which became co-authors); collected specimens in nature (as well as data about their ecology, behaviour and threats); analysed all available specimens in detail (including via loans or by visiting relevant museum collections); recorded all relevant morphological data, including (via specialised microscopic photography); presented new relevant distinctive features for the group and illustrated them in detail; wrote the first draft (including all graphics), incorporated co-authors suggestions and submit the manuscript for publication, including accepting and enacting the suggested minor revisions by the editor and reviewers.

Taxonomy and threats (Chapters 5 and 6)

Henriques, S. (pre-print) A survey of the *Loureedia* genus (Araneae, Eresidae) with a new species from Iran and the first assessment of illegal wildlife trade as a threat to the group. *https://doi.org/10.1101/2020.05.07.082891*.

The first author built upon previous literature reviews (published in the previous first-authored paper) when engaged by a student in the range country (who was invited to be a co-author but declined); analysed newly available specimens in detail (provided via loans and returned soon after); recorded all relevant morphological data, including (via specialised microscopic photography); used the new set of relevant distinctive features for the group to test the taxonomical hypothesis (published in the previous first-authored paper) and illustrated them in detail; conducted research about the online trade of these species, developed and experimental design to record and reported; wrote the first draft; submit the manuscript for pre-print.

Co-authorship

Taxonomy (Chapter 5)

Yamasaki, T., **Henriques, S.**, Phung, L. T. H., & Hoang, Q. D. (2018). Redescription of the sole species of the enigmatic solifuge genus *Dinorhax* Simon, 1879 (Solifugae: Melanoblossiidae) in Southeast Asia. The Journal of Arachnology, 46(3), 498-507.

I helped to review and interpret the biogeographical and historical context of the original literature about this species; provided insights on the group’s morphology from my museum visits and specimen analysis (in particular at the National Museum of Natural History, Paris and Senckenberg Museum, Frankfurt); advised about potential avenues of research in the group and its meaningfulness (in particular to test the “Wallace line” which this species seems to break); reviewed the original results and its interpretation, as well as the original draft manuscript, to which I provided input on technical/scientific content and English grammar.

Spider sRLI (Chapter 4)

Seppälä, S., **Henriques, S.**, Draney, M. L., Foord, S., Gibbons, A. T., Gomez, L. A., … & Cardoso, P. (2018). Species conservation profiles of a random sample of world spiders I: Agelenidae to Filistatidae. Biodiversity data journal, (6).

Seppälä, S., **Henriques, S.**, Draney, M. L., Foord, S., Gibbons, A. T., Gomez, L. A., ... & Cardoso, P. (2018). Species conservation profiles of a random sample of world spiders II: Gnaphosidae to Nemesiidae. Biodiversity data journal, (6).

Seppälä, S., **Henriques, S.**, Draney, M. L., Foord, S., Gibbons, A. T., Gomez, L. A., ... & Cardoso, P. (2018). Species conservation profiles of a random sample of world spiders III: Oecobiidae to Salticidae. Biodiversity Data Journal, (6).

Seppälä, S., **Henriques, S.**, Draney, M. L., Foord, S., Gibbons, A. T., Gomez, L. A., ... & Cardoso, P. (2018). Species conservation profiles of a random sample of world spiders IV: Scytodidae to Zoropsidae. Biodiversity Data Journal, (6).

I co-led the IUCN Red List workshop at the 20th International Congress of Arachnology (Colorado School of Mines, Golden, Colorado); leading the section on tools and resources for geographical data mining, record mapping and relevant metric calculations (like EOO and AOO).

Invertebrate conservation (Chapter 6)

Harvey, J. … **Henriques, S.** … & de Kroon, H. (2020). International scientists formulate a roadmap for insect conservation and recovery. Nature Ecology & Evolution: 1-3.

Akçakaya, R.; Hochkirch, A.; Bried, J.; De Knijf, G.; **Henriques, S.**, Simaika, J.; van Grunsven, R. (In press) Assessing Invertebrate Species with the IUCN Red List Process, Journal of Insect Conservation

Threats to spiders and those who study them (Chapters 4 and 6)

Böhm, M., **Henriques, S.**, Hochkirch, A, and Rodríguez, J. (2020) Conservationists deserve protection. Science 367 (6480): 861-861.

Milano, M; Blick, T, Cardoso, P.; Chatzaki, M.; Fukushima, C.; Gibbons, A.; **Henriques, S.**; Macías-Hernández, N.; Mammola, S.; Nentwig, W.; Nolan, M.; Pétillon, J.; Polchaninova, N.; Rezac, M.; Sandström, J.; Smith, H.; Wiśniewski, K.; Isaia, M. (submitted) Spider conservation in Europe. Biological Conservation

Fukushima C, Mendoza JI, West RC, Longhorn SJ, Rivera E, Cooper EWT, Hénaut Y, **Henriques S**, Cardoso P (2019) Species conservation profiles of tarantula spiders (Araneae, Theraphosidae) listed on CITES. Biodiversity Data Journal 7: e39342. *https://doi.org/10.3897/BDJ.7.e39342*

Branco, V. V., **Henriques, S.**, Rego, C., & Cardoso, P. (2019). Species conservation profiles of spiders (Araneae) endemic to mainland Portugal. Biodiversity Data Journal, 7.

Fukushima, C., Mendoza, J., West, R., Longhorn, S., Rivera Téllez, E., Cooper, E.W.T., **Henriques, S.** & Cardoso, P. 2019. *Aphonopelma pallidum* (amended version of 2019 assessment). The IUCN Red List of Threatened Species 2019: e.T66080948A148679253. http://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T66080948A148679253.en.

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Outhwaite, C.; Chapman, A.; Bridle, J.; Metcalfe, J.; **Henriques, S.**; Franklinos, L.; Ceausu, S.; Newbold, T.; Mace G. (2020) UK Parliament, Call for evidence on Biodiversity and Ecosystems. Biodiversity and Ecosystems enquiry.

As technical advisor and contributor

Threats (Chapters 4 and 6)

The Parliamentary Office of Science and Technology (2020) UK Insect Decline and Extinctions. Available at *https://post.parliament.uk/research-briefings/post-pn-0619/*

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Chapter 1

Introduction

"...as we know, there are known knowns; there are things we know we know.
We also know there are known unknowns;
that is to say we know there are some things we do not know.
But there are also unknown unknowns –
the ones we don't know we don't know."
Donald Rumsfeld – February 12, 2002 (while United States Secretary of Defence).

1.1. The biodiversity crisis

1.1.1. An unnatural history

Biologists have been concerned about the impact of human activities on the natural world at least since the 1800s, when trailblazer explorer Marianne North recounted her visit to the majestic redwoods of California, saying "I was very anxious to see some of the redwood forest. They had been so destroyed that it was not easy to get to them…They were gradually sawing them up for firewood, and the tree would soon be extinct. It is invaluable for many purposes, and it broke one's heart to think of man, the civiliser, wasting treasures..." (North 2011).

The key issues raised in this account contain all the features that would define the conservation biology movement for the next century, as North was not only concerned about the loss of the redwood's economic importance but also its intrinsic natural value as a "treasure". She had no doubt about assigning responsibility for this potentially irreversible loss to "man, the civiliser" (North 2011).

Famous naturalist Alfred Russel Wallace wrote in 1863 that the modern naturalist looks at "every species of animal and plant now living as one of the individual letters which go to make up one of the volumes of our Earth's history; and, as a few lost letters may make a sentence unintelligible, so the extinction of numerous forms of life which the progress of cultivation invariably entails will necessarily obscure this invaluable record of the past" (Drew 2011). Wallace also emphasised the "invaluable" importance of preserving species for non-economic gains, and human responsibility towards sustainable development, particularly in food production, which he called the threat caused by the “progress of cultivation”.

These concerns about sustainable development by both North and Wallace about protecting nature were reiterated by perhaps the most influential conservation pioneer, George Perkins Marsh, in his seminal work on the protection of natural landscapes and species sensitivity to the “violence of human force” (Marsh 1864). We now know that this force emerged much earlier than the 1800s and that the loss of natural resources has a long link to our species, as the palynological and archaeological records attest to significant levels of deforestation dating as far back as the Neolithic period (e.g. Hajar et al. 2010).

1.1.2. How we got here

Conservation biology has come a long way since its early pioneers; however, framing “nature for itself” would remain a main feature of the movement for a century, throughout the 1960s and 70s, with a key focus on species, wilderness, and protected areas. The scientific community would only come together for the first time in 1978, amid concerning predictions of significant loss of tropical forests and species extinctions, when an international group of scientists called on the US government “to support original research, both theoretical and applied, in the area of conservation biology”, and called on the world’s governments to protect the “remnants of natural habitat in tropical countries’’ (Douglas 1978; Soulé & Wilcox 1980). This was the beginning of the strong connection between conservation and policy goals, which is central to this thesis and is an intrinsic part and an ongoing driver of the field.

Michael Ellman Soulé, one of those present at this seminal 1978 conference, would set the standard of how the field should be framed, writing that “*although crisis oriented, conservation biology is concerned with the long-term viability of whole systems*” (Soulé 1985). The concerns highlighted then have been echoing since, but how these concerns have been framed has changed.

These events would trigger change towards a framing of “nature despite people” with a key focus on extinction, threats (e.g. habitat loss, pollution, overexploitation) and threatened species in the 1980s and ‘90s (Mace 2014). One of the most recurrent terminologies of this thesis and the field overall was established in this decade, encompassing life on Earth in all its levels of complexity, from genes to ecosystems, and their interconnectivity in a single word: *biodiversity* (Wilson 1988). The term *biodiversity* aimed to go beyond popularising what conservation aims to protect to drawing the U.S. Congress's attention to the "species extinction crisis" (Takacs 1996).

The key focus on extinction and threats seen in the 1980s and '90s (Mace 2014) also led E.O. Wilson to create the acronym HIPPO as a list of major threats to biodiversity, standing for Habitat destruction, Invasive species, Pollution, Population growth and Overharvesting (Wilson 2002). Despite the still unresolved nature of these threats, or perhaps because they seem unresolvable under past frameworks, there was a new shift in framing onto "nature for people", with a key focus on ecosystems, ecosystem services and economic values in the early 2000s, and more recently, a reframing towards "people and nature", with a key focus on environmental change, resilience, adaptability and socio-ecological systems (Mace 2014). The differences between these frameworks may appear stark, but none of them has ever truly disappeared, and instead, many have often coexisted side by side (Mace 2014). Today, what we see is a set of diverse approaches that encompass these views and their approaches to nature conservation (Sandbrook et al. 2019).

In 1988, the United Nations Environment Programme (UNEP) convened a group of experts on biodiversity to explore the development of an international convention on biological diversity; this working group became known as the Intergovernmental Negotiating Committee. It met at the Nairobi Conference in May 1992 to present an agreed text for the Convention on Biological Diversity (CBD 2004). In June 1992, at the United Nations Conference on Environment and Development in Rio de Janeiro, often called "Earth Summit", the CBD was proposed and soon after ratified. At the same meeting, a declaration on environment and development was made towards the sustainable management of forests, named Agenda 21, which was adopted by more than 178 Governments (United Nations 1992; Sitarz 1993). The CBD entered into force the following year, 1993, and the first session of the Conference of the Parties (COP) was scheduled for 1994 in the Bahamas. Its three main goals were the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of benefits arising from genetic resources (CBD 2002, 2004, 2010b, a, 2020a).

A decade later, in 2002, representatives of 190 countries met at the Johannesburg World Summit on Sustainable Development, building on Agenda 21, to ratify several biodiversity targets, including “a significant reduction of the current rate of biodiversity loss at the global, regional, and national level” by 2010 (CBD 2002), an important target that tragically was not met (CBD 2010b).

In the interim, there were renewed attempts to galvanise society as growing evidence emerged from the scientific community that the biodiversity crisis severely affected ecosystem services, on which human well-being also relies (Duraiappah et al. 2005). The CBD parties met again in Japan in 2010 and committed to implementing a Strategic Plan for Biodiversity 2011–2020, progress toward which is measured by the 20 Aichi Biodiversity Targets (CBD 2010a). Unfortunately, by its mid-term, we were not on track to meet the Aichi Targets (Tittensor et al. 2014), and the following year, renewed attempts were made to incorporate these targets into the United Nations Millennium Development Goals, in particular goal 7 focused on environmental sustainability (United Nations 2015).

Yet by 2020, although some progress had been made, not a single target was fully met (CBD 2020a). Although some targets under Strategic Goal B, “Reduce the direct pressures on biodiversity and promote sustainable use”, were partially met, i.e., target 9 (CBD 2020a), for the purposes of this thesis, it is important to note that target 12 that aimed at “*preventing the extinction of known threatened species and improving or sustaining their conservation status, particularly of those most in decline*” was sadly not met (see chapter 2 for more details).

In the interim, the UN General Assembly met in 2015, and the previously mentioned Millennium Development Goals were evaluated. New goals were set out, the 17 Sustainable Development Goals (SDGs), framed by UN Secretary-General Ban Ki-moon as “our shared vision of humanity and a social contract between the world’s leaders and the people. They are a to-do list for people and planet, and a blueprint for success” (United Nations 2015). The most relevant of these SDGs, for the context of this thesis, are Target 14 and Target 15, which aim to “Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss” (United Nations 2020).

In 2019, the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) reported that nature is declining globally at unprecedented rates based on a review of approximately 15,000 scientific and government sources (IPBES 2019). The 196 parties to the CBD are expected to meet in May 2021 to draw up a post-2020 global framework of biodiversity targets towards the 2050 vision of “Living in harmony with nature” (CBD 2020a).

1.1.3. Biodiversity indicators

The international agreements described above established a series of *biodiversity targets*. Such targets are monitored using a wide range of *biodiversity indicators*. These are crucially important communication tools that summarise complex environmental issues, functioning as a bridge between policy-making and science. Biodiversity indicators are, in essence, aggregate statistics that were selected for both their scope and scalability, as well as their ability to detect and easily convey progress towards the goals we want to achieve. These face many challenges, including how to reconcile different indicators (Hill et al. 2016; Newbold et al. 2019) and, perhaps more importantly, how to convert them to conservation action and meet conservation goals (CBD 2020b), but inaccessibility is not one of these challenges, as they have been summarised and made available via the Biodiversity Indicators Partnership (BIP), which is a global initiative to promote the development and delivery of biodiversity indicators, which includes maintaining an updated list of the targets they are most suitable to measure success towards (BIP 2020).

However, despite containing more than 60 biodiversity indicators (BIP 2020), gaps have been identified in the 20 Aichi Targets, where targets 2, 3, and 15 had no formally recognised indicator (Mcowen et al. 2016). Some of these gaps have recently been addressed, with primary indicators now available for targets 3 and 15. However, none is yet available for target 2 (BIP 2020), and not all different levels of biodiversity have thus far been suitably represented, such as ecosystems (Keith et al. 2015) or genes (Hoban et al. 2020).

Biodiversity indicators are based on a number of metrics and datasets, including *The World Database on Protected Areas*, which lists nationally and internationally protected areas (UNEP-WCMC et al. 2018), or the IUCN Red List of Threatened Species, which is based on a set of criteria (Table 1.1.) to assess extinction risk (IUCN 2020). The

IUCN Red List informs the Red List Index (RLI) (Butchart et al. 2007), the same way local biodiversity surveys combined with global land-use data inform the Biodiversity Intactness Index (BII) (Newbold et al. 2016), or vertebrate trends inform the Living Planet Index (LPI) (Collen et al. 2009). Which, although sharing similar goals, are, in fact, distinct but complementary biodiversity indicators.

Table 1.1 - Summary of the IUCN Red List criteria, adapted from the IUCN Red List Criteria Summary Sheet available online (https://www.iucnredlist.org/resources/summary-sheet)

A. Population size reduction *(measured over 10 years or 3 generations, whichever is the longest)*			
	Critically Endangered	**Endangered**	**Vulnerable**
A1	≥ 90%	≥ 70%	≥ 50%
A2, A3, A4	≥ 80%	≥ 50%	≥ 30%
A1 Population reduction observed, estimated, inferred, or suspected in the past where the causes of the reduction are clearly reversible AND understood AND have ceased. A2 Population reduction observed, estimated, inferred, or suspected in the past where the causes of reduction may not have ceased OR may not be understood OR may not be reversible. A3 Population reduction projected, inferred or suspected to be met in the future (up to a maximum of 100 years) *[(a) cannot be used for A3].* A4 An observed, estimated, inferred, projected or suspected population reduction where the time period must include both the past and the future (up to a max. of 100 years in the future) and where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.			(a) direct observation (b) an index of abundance appropriate to the taxon (c) a decline in AOO, EOO and/or habitat quality (d) actual or potential levels of exploitation (e) effects of introduced taxa, hybridisation, pathogens, pollutants, competitors or parasites.
B. Geographic range *(B1 and/or B2 and at least 2 of (a)-(c))*			
B1 (EOO)	< 100 km2	< 5,000 km2	< 20,000 km2
B2 (AOO)	< 10 km2	< 500 km2	< 2,000 km2
(a) Severely fragmented OR number of locations	= 1	≤ 5	≤ 10
(b) Continuing decline observed, estimated, inferred or projected in any of: (i) EOO; (ii) AOO; (iii) area, extent and/or quality of habitat; (iv) number of locations or subpopulations; (v) number of mature individuals			
(c) Extreme fluctuations in any of: (i) EOO; (ii) AOO; (iii) number of locations or subpopulations; (iv) number of mature individuals			
C. Small population size and decline *(number of mature individuals and at least one of C1 or C2)*			
Number of mature individuals	<250	<2,500	<10,000
C1. An observed, estimated or projected continuing decline of at least (up to a max. of 100 years in future):	25% in 3 years or 1 generation (whichever is longer)	20% in 5 years or 2 generations (whichever is longer)	10% in 10 years or 3 generations (whichever is longer)
(a) (i) Number of mature individuals in each subpopulation	≤ 50	≤ 250	≤ 1,000
(ii) % of mature individuals in one subpopulation =	90–100%	95–100%	100%
(b) Extreme fluctuations in the number of mature individuals			
D. Very small or restricted population			
D. Number of mature individuals	<50	<250	D1. <1,000
D2. Restricted AOO or number of locations	N/A	N/A	D2. typically: AOO < 20 km2 or number of locations ≤ 5
E. Quantitative Analysis *(up to a maximum of 100 years)*			
Probability of extinction	≥ 50% in 10 years or 3 generations, whichever is longer	≥ 20% in 20 years or 5 generations, whichever is longer	≥ 10% in 100 years

The BII estimates the average abundance of species in an area relative to what those populations could be under no human pressure (Newbold et al. 2016). It solely covers the world's terrestrial environments but can report at multiple policy-relevant scales (from global to regional or even local) and incorporates a global database of local biodiversity surveys on a wide range of taxa, including invertebrates, providing important estimates of human activities impact on the intactness of local biodiversity, and how this may change over time (Newbold et al. 2016). The BII informs important targets within IPBES and CBD (BIP 2020) and has recently been estimated to be, on average, below ~60% in terms of species abundance and below ~50% in terms of species richness when compared to the baseline of these habitats (Sanchez-Ortiz et al. 2019).

The LPI calculates the average rate of population change through time, using generalised additive modelling to determine the underlying trend in each population time series (Collen et al. 2009). It is currently solely based on population trends of vertebrate species. Still, it has a global coverage of terrestrial, freshwater and marine environments, and it has recently started to include analysis of invertebrates and plants (WWF 2020). The LPI is based on thousands of population time series collected from monitored species from around the world, and valuable strides have been made to facilitate how data are incorporated into this indicator (Cornford et al. 2020), which promises to increase its scope and quality even further. It can be aggregated into different scales, including at the national level (McRae et al. 2007) or aimed at specific groups (Saha et al. 2018) and the global LPI method of aggregation accounts for a number of factors and potential biases, assigning a higher weight to trends from more species-rich systems, realms and groups (McRae et al. 2017). Because it can accurately and rapidly track changes in vertebrate trends, it is particularly suitable to inform policy making (Collen & Nicholson 2014). It is, therefore, currently used on several important targets, such as CBD or the Aichi Targets (BIP 2020). The latest LPI report showed that there was an average decline in abundance of 68% between 1970 and 2016 (WWF 2020), and although there is considerable complexity behind this value (Freeman 2020; Leung et al. 2020), over the same period, freshwater populations were estimated to have declined by 84% on average, and Latin America & Caribbean populations declined 94% on average (WWF 2020).

Complementary to the BII and LPI, the RLI is based on the IUCN Red List of Threatened Species, which assesses species extinction risk into standardised categories based on several criteria, which include but are not restricted to species abundance, range and trends (IUCN Standards and Petitions Subcommittee 2016). This is crucial because abundance data are not readily available for all species, and the IUCN Red List allows the analysis of a wider range of known symptoms of extinction risk, such as reduction in Extent of Occurrence or Area of Occupancy (IUCN 2020). The IUCN Red List covers all ecosystems and is not restricted to specific taxonomic groups, which are some of the reasons why the RLI is one of the most important biodiversity indicators. The RLI is currently the primary indicator of a number of important biodiversity goals within the SDG, IPBES, CBD or the Aichi Targets (BIP 2020) and how best to use it towards assessing the extinction risk of megadiverse groups will be the aim of this thesis.

1.1.4 Introducing the Red List Index

The RLI presents an aggregate assessment of the extinction risk of a group of species. It combines IUCN Red List categories (e.g. LC, VU, etc. see below), weighting them equally into a single number between 1 (all species are Least cConcern) and 0 (all species are extinct). It can be applied to all species assessments within a particular taxonomic group (e.g. all species of mammals) or to all species within a particular subset of interest (e.g., all species of mammals that are under human use or trade) (Butchart et al. 2004; Butchart et al. 2005; Butchart et al. 2006; Butchart et al. 2007; Butchart 2008). This protocol enables the RLI to be analysed as a value in itself, to compare two different taxonomic groups, for example or can be set as a baseline towards which to monitor change over time. A much more detailed description of the methodological approach of this biodiversity indicator is also presented in chapters 2 and 3.

The RLI is currently used internationally (Butchart et al. 2007; BIP 2020) but has also long been used at a national or regional scale (Szabo et al. 2012; Juslén et al. 2013; Koyanagi & Furukawa 2013; Garcia-R & Di Marco 2020; Renjifo et al. 2020). This is a significant part of what makes the RLI such an important biodiversity indicator because it is at the national/regional level that most policy decisions are enforced and conservation action implemented. It has also been applied to particular species subsets within thematic levels, such as measuring the impacts of trade, invasive species (Butchart 2008), the impacts of the conservation work by a single organisation (Young et al. 2014) or as an indicator of habitat change (Juslén et al. 2016). A recent

RLI analysis of target bird species showed that the improvements in conservation status caused by successful control and management of use and trade are currently still outweighed by the deteriorating status of species suffering from unsustainable exploitation (Butchart 2008). Another analysis used an RLI approach to detect the impact of a conservation organisation on the extinction risk of target species, showing that conservation action can lead to a 67% increase in RLI in contrast to a 23% decline in a counterfactual RLI if no conservation action had taken place (Young et al. 2014).

This flexibility of the RLI to be implemented to targeted species subsets has placed the RLI as a primary global biodiversity indicator of Aichi target 4 (impact of human exploitation and internationally traded species), target 5 (forest specialist species), target 6 (impacts of fisheries and trends in target and bycatch species), target 8 (impacts of pollution), target 9 (impacts of invasive alien species), target 10 (reef-building corals), target 13 (wild relatives of domesticated animals), target 14 (pollinating species and species used for food and medicine), and importantly target 12: “by 2020, the extinction of known threatened species has been prevented and their conservation status, particularly of those most in decline, has been improved and sustained” (Balmford et al. 2005; CBD 2010b, a, 2020a, b).

1.1.5. Biodiversity indicator metrics

Until 1990, species listed according to their threats, as presented in the then available Red Data Books, were largely subjective making them neither replicable nor often reliable (Mace & Lande 1991). The foundations of the objective scientific assessment of extinction risk we have today were only made possible with the seminal work of Georgina Mace (Mace et al. 2008), leading to more than 120,000 species assessed under its criteria to date (IUCN 2020).

As mentioned in the two previous sections, 1.1.3 and 1.1.4, the RLI is focused on the analysis of all species within a target group based on their assigned IUCN Red List categories. Namely Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Extinct in the Wild (EW), and Extinct (EX), listed from the lowest extinction risk to the highest, where extinction has sadly already been recorded. When a species data availability are so poor that it might be anything between LC and Ex, it is placed in the Data Deficient (DD) category (IUCN Standards and Petitions Subcommittee 2016).

The extinction risk category a given species can be placed in is assessed based on five well defined criteria (Table 1.1.): *Criterion A* is mostly based on population size reduction, measured over 10 years or 3 generations (whichever is longer); *Criterion B* is mostly based on reduction in geographic range, measured as Extent of Occurrence and Area of Occupancy; *Criterion C* that is mostly based on small number of mature individuals and continuing decline; *Criterion D* that is mostly based on very small or restricted populations; and *Criterion E* that is mostly based on the probability of extinction under a quantitative analysis (IUCN Standards and Petitions Subcommittee 2016).

However, this is not the only such metric; the framing of “nature for itself” in the 1960s and particularly in the ’70s (Mace 2014) would lead to the first lists of species perceived as threatened in the United States. The Species Status Assessment was developed under the Endangered Species Act of 1973, which set the U.S. Fish and Wildlife Service with the responsibility to enforce the protection of taxa in the Endangered Species List, which assigns a set of Species Status Codes based on the species’ likelihood of becoming endangered throughout all or a significant portion of its range (USFWS 2020). In New Zealand, species have long been assessed under a national Threat Classification System, which is based on determining extinction risk and a number of other qualifiers, such as species being biologically sparse, within the category “Naturally Uncommon” (Townsend et al. 2008). It shares common aims and several common approaches to the system implemented in the US or The IUCN Red List of Threatened Species but is substantially different from either.

The IUCN Red List is the most widely used system globally. Still, even within regions like the US, where, as I mentioned, distinct metrics are available at a state level, the IUCN Red List might apply, such as the system enforced by the Florida Fish and Wildlife Conservation Commission, which since 1999 has used the IUCN criteria but under slightly different category names (Fish and Wildlife Conservation Commission 2020). Furthermore, in scientific research, the IUCN Red List has also been used to monitor the trend of some of New Zealand’s most iconic species (Garcia-R & Di Marco 2020). Therefore, although the IUCN Red List categories and criteria are not the only biodiversity indicators used today, their impact is clearly high and far-reaching.

We are in a drastic period of biodiversity loss, the likes of which has only been recorded five times in the fossil record, making the current biodiversity crisis a prelude to the sixth mass extinction, but one which this time is being caused by a single species, humans (Ceballos et al. 2015). Very conservative estimates suggest that the current species extinction rate is at least a hundred times faster than the natural background or pre-human rate (Ceballos et al. 2015) but that it may currently be a thousand times higher and is likely to reach ten thousand in the future (De Vos et al. 2015).

However, biodiversity indicators based on the number of extinctions have only recently been proposed. The reasoning behind such indicators is to drive policy action and facilitate policy agreements in a similar way that a 2°C global mean temperature change galvanised the climate change community (Rounsevell et al. 2020). Overall, biodiversity indicators are still based on what increases extinction risk, such as population decline, range reduction, etc. Towards driving action to prevent species from becoming extinct rather than detecting when extinction has already happened. It is not too late to reverse the biodiversity crisis if we act now (Leclère et al. 2020).

1.2. Analysing IUCN Red List data

Metrics that support biodiversity indicators vary and can originate from recorded extinctions (Ceballos et al. 2015; Rounsevell et al. 2020), population trends (WWF 2020), often from extinction risk assessments (IUCN 2020) or many other sources. However, biodiversity indicators are at the basis of informed decision making, because they are how we measure success or failure, and set the path ahead. It is therefore important to understand how the metrics that inform them have been used but also when they may be misinterpreted. In this section, we will focus on the metric that informs the RLI, The IUCN Red List of Threatened Species.

Currently, 128,918 taxa have been placed under the IUCN Red List (IUCN 2020), a remarkable achievement in itself. However, this represents approximately 5% of the world's 1.7 million recorded species out of a conservative estimate of 8.7 million existing species (Mora et al. 2011), lowering the number of assessments to less than 1% of Earth’s biodiversity. The IUCN Red List is mostly composed of non-random samples of particular species groups, which one should be careful to interpret as representative of the impact of threats at a global scale, as clear bias exists and has often been misunderstood (Akçakaya et al. 2006; Collen et al. 2016). I will discuss at length the merits and best approaches to a random sample of species; in this section, I will frame their value in the context of non-random approaches and what currently available IUCN Red List data can and cannot tell us.

Many summaries and basic analyses based on IUCN Red List data have been published over the years. For example, a comment piece by Maxwell et al. (2016) used comprehensively assessed groups on the IUCN Red List (Figure 1.1) to analyse the main threats to biodiversity globally. In their conclusions, they argued that conservationists should refocus their efforts from the anthropogenic climate crisis towards prevalent threats in the IUCN Red List. The "big killers" mentioned are indeed priorities; however, it also explicitly suggests climate change should be deprioritised as a conservation threat, which is an oversimplified view of the IUCN Red List data available. I will explain my reasoning for why this is the case in the paragraphs bellow.

The set of species analysed by Maxwell et al. (2016) cannot be expected to accurately represent Earth's ecological trait diversity (Chichorro et al. 2019), small and large, endotherms and ectotherms, nor low and high trophic levels, differences which will likely play a significant role in species extinction vulnerability to climate change (Aragón et al. 2010; Pearson et al. 2014; Thackeray et al. 2016).

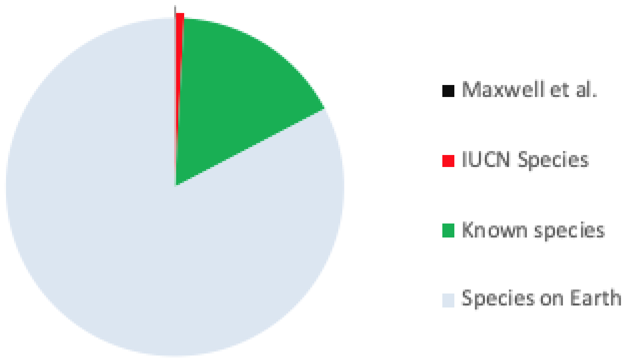

Figure 1.1 – Proportion of species analysed by Maxwell et al. (in black), when compared with the species assessed by IUCN Red List criteria at the time (in red), the number of known species (in green) and the number of predicted species on earth (in grey).

Historically, regions that have conducted more research on biodiversity have also gathered more knowledge on its threats, which differ from the threats affecting understudied regions, like deserts (Durant et al. 2012), where 81% of the target groups analysed by Maxwell et al. (2016) do not live (e.g. marine species), but an important habitat analysis towards measuring the impact of this threat as the velocity of climate change is expected to be faster in arid regions than in well studied temperate habitats (Loarie et al. 2009).

Non-random samples and low data availability are particularly relevant for impacts that are harder to quantify remotely via spatial data, such as invasive species. As well as towards detecting impacts that are felt in longer timeframes and have only recently become a research focus, such as climate change. 79% of scientific publications on climate change ‘multiple stressors’ and human vulnerability have been published after 2009 (Räsänen et al. 2016). This temporal availability of data is an important factor from an IUCN Red List perspective because emerging threats generate a distinction between earlier assessments that are less likely to mention climate change (e.g. amphibians). In contrast, more recent ones are more likely to incorporate it.

To this day, data on climate change impacts are not as available as data on other biodiversity threats that have been recognised and recorded for decades. Currently, up to 66% of amphibians, 70% of corals and 83% of bird species with high climate change vulnerability have not yet been listed in any of the IUCN Red List threatened categories (Foden et al. 2013) as it is important to note that vulnerability is determined by future exposures to climate change impacts and the IUCN Red List assessment focus on current extinction risk (IUCN, 2019).

Climate change threats are also not easily assessed because, contrary to the “big killers” mentioned in Maxwell et al. (2016), its impacts are rarely direct but instead act by exacerbating multiple interacting extinction drivers (Dukes & Mooney 1999). Although the IUCN acknowledged concerns that its criteria may not adequately allow to assess vulnerability to climate change (IUCN Standards and Petitions Subcommittee 2016), studies have shown that the IUCN Red List can indeed identify and predict climate change extinction risk (Pearson et al. 2014; Stanton et al. 2015). However, in practice, assessors previously found it challenging to assess as a threat because the criteria focus on proximate causes, while climate change often is a distal cause, and its impact is rarely direct but instead acts by exacerbating multiple extinction drivers (Brook et al. 2008).

This gap was set to change with the release of specific IUCN guidelines to incorporate climate change (IUCN Standards and Petitions Subcommittee 2016); therefore, what we find in the IUCN Red List data sets of several taxonomic groups (e.g. amphibians) is that more recent assessment already incorporate climate change as a threat, whereas assessments made several years ago, prior to published guidelines, do not. Currently, climate change is recognised as one of the most prominent future threats in several groups (e.g. Böhm et al. 2020), and its frequency in the IUCN Red List is likely to increase in future assessments (supplementary material S6).

However, challenges still exist as distal causes are still not usually considered in IUCN extinction risk assessments and, consequently, are often not mentioned (Trull et al. 2018). A particularly interconnected example of the difficulty of including distal causes in the IUCN Red List is invasive species, as regions previously unsuitable to non-native species might become susceptible to receiving and sustaining invasive species under future climate change scenarios (Dukes & Mooney 1999; Hulme 2017). Therefore, threats such as deforestation remain more readily chosen and reported because they are more detectable, including via remote sensing (Buchanan et al. 2008).

This argument is made even more poignant when we consider that the IUCN Red List is currently not set to be a suitable sample to detect how impactful invasive species are on global biodiversity. A clear example of this is the work of Gurevitch and Padilla (2004), which concluded that the impact of invasive species in the declines and extinctions of species is unproven because only 6% of the taxa in the IUCN Red List at the time were threatened with extinction as a result of invasion by alien species. This analysis neglects that threat information is not required in Least Concern species and that the IUCN Red List was never conceived to be representative of global impacts of a particular threat, a common misconception shared with the previous example (Maxwell et al. 2016). However, although Gurevitch and Padilla (2004) research and its methodological shortfalls have been scrutinised (Clavero & García-Berthou 2005), Maxwell et al. (2016) have not, and it is important to note that these publications are impactful as according to Google Scholar, Gurevitch and Padilla (2004) has been cited 1587 times by 2020 while Maxwell et al. (2016) was quoted 737 times in the last four years.

Oversimplified views of the data available in the IUCN Red List are a recurrent issue of underrepresentation but also misrepresentation. A recent paper by Scheffers et al. (2019) concluded that 24% of terrestrial vertebrates are globally traded based on the IUCN Red List and CITES listed species. One of the components of Scheffers et al. (2019) analysis was IUCN Red List assessments that have been tagged with “hunting and collecting terrestrial animals” as a threat under the subcategory “intentional use” (Scheffers et al. 2019). Beyond the limitations of species representation in the IUCN Red List, it is currently not compulsory to provide code threat information for Least Concern species. It might, therefore, go unrecorded for these species even when present (IUCN 2013). The authors recognise this would be important to encourage, but it is a caveat they did not tackle in their analysis. This publication also neglects that CITES does not solely list traded species but can also include those that resemble or are closely related to traded species (CITES 2020). Therefore, how these two issues might have impacted the 4,545 sp. identified as traded in the IUCN Red List but not listed in CITES and 723 sp. in CITES but not in the IUCN Red List (Scheffers et al. 2019) remains unknown.

Importantly, well-regulated trade can be sustainable (Hutton & Leader-Williams 2003). Still, because trade volume was not analysed, the identification of hotspots by Scheffers et al. (2019) was set to represent the diversity of intentionally used species. This approach does not reflect these practices' impact or overall magnitude on these species, making this particular analysis unfit to set conservation priorities. According to the authors themselves, it failed to detect areas where a high abundance of traded birds is known to take place, such as Indonesia. It is worth noting that more suitable approaches have already been made, solely focusing on trade as a major threat in comprehensively assessed groups while considering the caveats mentioned in this section (Marsh et al. 2020).

The IUCN Red List is one of the most important tools available for conservationists, but the data it contains has often been misinterpreted (Akçakaya et al. 2006; Collen et al. 2016). Assessments are currently scarce, taxonomically and geographically biased, and emphasise short-term threats, whereas climate change, for example, has mostly long-term consequences; that was why other ways to assess its impact were devised (Foden & Young 2016). Defining research and conservation priorities is such an important endeavour that it is crucial to ensure we do not under-prioritise the most challenging threats due to bias on the way data are collected, lack of data on their impacts or simply lack by not using the relevant data to detect them. These biases are complex and interconnected, but many derive from the fact that the current IUCN Red List is currently a non-random sample of global biodiversity. What would a random sample of species be able to tell us?

1.3. The sampled Red List Index

1.3.1. Diversity

As I have shown, despite the remarkable ongoing efforts to assess species extinction risk under IUCN Red List Categories and Criteria, the currently listed species are still a small percentage of global biodiversity (Figure 1.1). This is reflected in the RLI because it is based on the weighted IUCN Red List categories of all species within a particular taxonomic group, and very few non-vertebrate groups have had their extinction risk assessed to enable the implementation of its protocol.

There are still many challenges in understanding why some clades are more diverse than others (Wiens 2011). Still, the fact is that Earth today has vastly different species richness patterns across groups, with invertebrates comprising more than 75% of known species (Mora et al. 2011). Due to this disproportionate representation of invertebrates in the tree of life, it is not surprising that despite early attempts (Temple & Wiens 1989), less diverse groups, including birds, have repeatedly proven to be poor indicators of biodiversity loss in a variety of ecosystems (Estrada & Rodríguez-Estrella 2016; Ruaro et al. 2016; Siddig et al. 2016; Rodríguez-Estrella et al. 2019), while arthropod groups, including spiders, have been reliably used to indicate disturbances in the environment (Maelfait & Hendrickx 1997; McGeogh 1998; Rainio & Niemelä 2003; Avgın & Luff 2010), reflecting the responses of other species and overall biodiversity (Pearce & Venier 2006; Maleque et al. 2009; Borchard et al. 2014). While less diverse than arthropods, Plants and Fungi also have a high potential as indicators but have equally been underutilised.

Currently, the groups that have been comprehensively assessed and are able to implement an RLI represent a small percentage of life on earth, mostly vertebrates, namely amphibians, birds, and mammals (IUCN 2020). Only one small group of plants, cycads, has been included in this indicator, and only one marine invertebrate group, reef-building corals (IUCN 2020). Meanwhile, megadiverse groups that do represent large portions of known species are too speciose to allow for a timely assessment of all their species under current financial support systems, and even the most basic data about the majority of them is yet to be compiled (Pimm et al. 2014). Megadiverse groups, like the plant order Asparagales (containing orchids) or the arthropod order Araneae (containing house spiders), share two common features: they contain, by definition, a large number of taxa, and perhaps as a consequence, very little is known about most of those species (Cracraft & Grifo 1999).

It should be a concern of the entire conservation community that we are yet unaware of the extinction risk of the vast majority of species during our current extinction crisis (Dirzo et al. 2014; Pimm et al. 2014; Sullivan & Ozman-Sullivan 2020). Although there are many reasons why megadiverse groups have not been assessed (Cardoso et al. 2011; Cardoso et al. 2012), it is the fact that they have not that makes the IUCN Red List representativeness so low (Figure 1.1). However, for these megadiverse groups, or for any others that might not have been comprehensively assessed yet but would benefit from the RLI, a sampled approach has been proposed to ensure that those selected species extinction risk assessments are representative of the entire group's extinction risk trend, hence designated as the sRLI (Baillie et al. 2008).

This sampled approach has been used in several plant groups, namely pteridophytes, bryophytes, monocots and legumes (Brummitt et al. 2015), Ascomycota fungi (Minter 2011), and animal groups such as dragonflies (Clausnitzer et al. 2009), fish (Baillie et al. 2010; Hoffmann et al. 2010), reptiles (Böhm et al. 2013) and freshwater molluscs (Böhm et al. 2020). Work is in progress for butterflies (Lewis & Senior 2011), dung beetles (Collen et al. 2012) and grasshoppers (Hochkirch 2019 pers. comm.). This substantial increase in groups implementing the RLI via a sampled approach is a testament to its impact. While valuable efforts have been made to accelerate species assessments (Bachman et al. 2020), many challenges remain and are particularly hard to overcome in megadiverse groups, several of which will be addressed in chapters 2 and 3.

1.3.2 Spiders: Megadiverse and poorly studied

Spiders are the most diverse group of predators on earth (Wise 1995), have long been recognised for their extraordinary ecological significance (Riechert 1974) and are one of the major groups in terms of abundance and biomass (Koltz et al. 2018). They are also ubiquitous in terrestrial ecosystems and cover a wide range of habitats and ecological niches, from leaf litter to tree canopies, from the top of the highest mountains in the world to deep caves, from the hottest deserts to the coldest ones (Wise 1995).

Among invertebrates, arthropods are by far the most diverse group (Mora et al. 2011), but the main conservation focus has been on target insect groups. In contrast, other arthropod groups, such as spiders, are still underrepresented in conservation regulations, even within wealthy regions such as Europe (Milano et al. in press). 48953 species of spiders are currently recognised worldwide (World Spider Catalog 2020). In a taxonomic group where a single publication can describe 42 new species (Miglio et al. 2020), it is not surprising that species numbers have been growing year by year with 814 new species described in 2018 alone and 834 in 2019 (World Spider Catalog 2020).

For a group to implement an sRLI, the original authors of this approach put forward three requirements: 1. there must be a complete or near complete global species list (preferably including all published names and distributional information); 2. the species list must have a person or organisation updating the list, with mechanisms in place to assure it’s continuation; 3. the list must contain over 1,500 species, as this was the minimum sample size observed in an analysis of comprehensively assessed groups at the time (Baillie et al. 2008). This proposed sample size will be re-analysed in detail in chapter 2.

With these sRLI requirements in mind, within the most diverse group of organisms, arthropods (Mora et al. 2011), arachnids stand out for being remarkably well catalogued. From the 114 275 described species of arachnids (Sullivan & Ozman-Sullivan 2020), updated lists exist for several of its orders, such as opilionids with over 6500 species (Kury et al. 2020), pseudoscorpions with more than 3,600 species (Harvey 2003) and scorpions with more than 2000 species (Fet et al. 2000; Dupré 2010; Rein 2017), as well as a published catalogue of the minor arachnid orders (Harvey 2003), and importantly the megadiverse order of spiders with 48953 accepted species (World Spider Catalog 2020). These resources jointly cover all arachnids except for Acari, for which only the Oribatid catalogue has been compiled (Schatz

2004). Therefore, an arachnid order that has also undergone a concerning decline (Napierała et al. 2018; Sullivan & Ozman-Sullivan 2020) should also be ranked highly in conservation priorities.

The updated version of the World Spider Catalogue is freely available online (World Spider Catalog 2020), with all the currently accepted species' names and the geographical regions where the species are known to exist. It provides access to all species original publication and a number of relevant bibliographical resources. It has been kept up to date for decades, and measures are in place to ensure that it will continue.

Such a complete information source is unparalleled in megadiverse groups, making this an interesting case study for the sRLI method. When also considering this group's ubiquity in terrestrial ecosystems, the breadth of habitats spiders occupy and their overall ecological importance (Riechert 1974; Wise 1995), it is not surprising that experts have identified spiders as a priority group for IUCN Red Listing (Gerlach et al. 2014).

1.4. Aims, scope and outlook

Since the inception of the sRLI, several groups have been comprehensively assessed, such as mammals, reef building corals and cycads, or have been reassessed, such as birds. As these new sources of data became available, in chapter 2 we set out to investigate whether previous results still hold true under a wider taxonomic range and under the current policy targets, and whether that impacts sample size. In chapter 3 we broaden the scope of this approach and test how ongoing efforts to implement the sRLI, set to detect trend direction over a period of ten years, might inform us about the groups' overall proportion of threatened species now. In chapter 4 we implement our findings towards the first sRLI assessment of a megadiverse invertebrate group, spiders. In chapter 5 I address taxonomy as one of the root causes of data deficiency and propose mechanisms for anyone facing the same issues. These five data-driven chapters are then followed by a final synthesis chapter, where we summarise the findings, discuss them in light of current policy and present potential paths forward.

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Chapter 2

Accelerating the monitoring of global biodiversity: Revisiting the sampled approach to generating Red List Indices

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Abstract

Given the current biodiversity crisis, pragmatic approaches to detect global conservation trends across a broad range of taxa are critical. A sampled approach to the Red List Index (RLI) was proposed, as many groups are highly speciose and poorly known.

However, a decade after its conception, the recommended 900 species sample was only implemented in six groups and trend data are available for none, potentially because this sample is unfeasibly high.

Using a broader data set we show that when re-assessments are conducted every 10 years (the time limit for valid assessments), 400 species is sufficient to detect RLI trend direction. Correctly detecting changes in trends requires larger samples of 900 species (11,000 in some cases).

Sampled assessments can accelerate biodiversity monitoring and complement current metrics. Still, time-period between assessments and the approaches' purpose should be carefully considered, as there is a trade-off between sample size and the resulting indices.

2.1 Introduction

The International Union for Conservation of Nature (IUCN) Red List of Threatened Species (hereafter Red List) is the world's most comprehensive repository of conservation assessments, containing information on the extinction risk of more than 98,500 species (IUCN 2019). However, this only encompasses 5% of the world's 1.8 million recorded species, and less than 1% of Earth’s estimated biodiversity (Mora et al. 2011). Therefore, our understanding of extinction risk at a global scale may be biased. A pragmatic approach to determining extinction risk (and its trends) for these unknown majority is of critical importance (Collen & Baillie 2010).

The IUCN Red List applies quantitative criteria (Mace et al. 2008) to place species in one of seven ranked extinction risk categories (IUCN 2012): Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Extinct in the Wild (EW) and Extinct (EX). For the taxonomic groups where all species have been assessed, assigning incremental weights to the ordinal ranks of threat categories (from LC = 0 to EX = 5) allows a Red List Index (RLI) to be calculated, while a repeat assessment of these species allows for a measurement of the entire group’s extinction risk trend (Butchart et al. 2004; Butchart et al. 2007; Butchart et al. 2010).

RLIs have been calculated for all birds (Butchart et al. 2004), mammals (Hoffmann et al. 2010; Hoffmann et al. 2011), amphibians (Hoffmann et al. 2010), reef-building corals (Carpenter et al. 2008), and cycads (United Nations 2015). This coverage carries a number of taxonomic, ecological and geographical biases: vertebrates are relatively well represented, whereas invertebrates (Collen et al. 2012), plants (Brummitt et al. 2015) or desert species (Durant et al. 2012) among others, are far less so, reflecting resource biases and the challenges of comprehensively assessing very speciose groups. As the Red List Index is widely used for monitoring progress against globally agreed biodiversity targets and sustainable development (e.g. Tittensor et al. 2014; United Nations 2015, 2018; Díaz et al. 2019), this raises concerns that our reporting on biodiversity loss may not adequately represent trends across taxa and ecoregions.

To tackle these challenges, a sampled approach to the Red List Index was proposed to monitor progress towards the 2010 biodiversity target to “significantly reduce the rate of loss of biodiversity” (Baillie et al. 2008). Using Red List data for all birds measured at

4 or 6 year intervals (from 1988-2004) and amphibians (1980-2004), the authors assessed at which sample size there was a 5% probability of falsely detecting a positive index slope, when the true trend of those two groups was negative (Baillie et al. 2008). This led to a recommended sample of 900 non-Data Deficient (henceforth referred as non-DD) species (Baillie et al. 2008). Such a sampled approach was designed with the intention of undertaking repeated assessments over time. However, to date, only baseline assessments have been completed, including for dragonflies (Clausnitzer et al. 2009), bony fish (Baillie et al. 2010), reptiles (Böhm et al. 2013) and several plant groups, namely pteridophytes, bryophytes, monocots and legumes (Brummitt et al. 2015). Work is underway for butterflies (Lewis & Senior 2011), freshwater molluscs, dung beetles and spiders (Seppälä et al. 2018a, b, c, d). However, given that in the decade following its inception only six groups have completed a first sampled assessment, and only reptiles have a reassessment close to publication to estimate their extinction risk trend, it is clear that this protocol is currently challenging to implement.

In the decade since the sampled approach was first proposed, more data have become available to assess recommended sample sizes. Firstly, new comprehensively assessed datasets have been completed for mammals, corals and cycads. Secondly, additional comprehensive reassessments have been produced for birds (in 2008, 2012, 2016), adding three new data points to the original analyses. Many species have also had their previously published Red List categories updated retrospectively, as newly acquired information has become available (IUCN 2012). Most importantly, the current policy context differs from the 2010 biodiversity target. The current Sustainable Development Goal 15 and Aichi target 12 aim, "By 2020, the extinction of known threatened species has been prevented and their conservation status, particularly of those most in decline, has been improved and sustained”. This would include measuring if RLIs continue to decline, are slowing in their decline, or are starting to increase.

Using a much larger and updated dataset of 23539 assessments, we investigate:

1) whether the originally proposed sample size by Baillie et al. (2008) remains appropriate;

2) how the length of time intervals between re-assessments affects the required sample size;

3) how our findings fit within the context of current and potential post-2020 biodiversity targets.

2.2 Materials and methods

2.2.1 Data collection

We compiled the IUCN Red List categories of taxonomic groups that have had all their species reassessed at least once (see supporting information S1). We recorded the length of time between these comprehensive assessments, hereafter referred to as inter-assessment period (e.g. an inter-assessment period of 12 years between 1996 and 2008), which are substantially different among taxa (see supporting information S1). We analysed each taxonomic group and inter-assessment period separately.

2.2.2 The RLI and its sampled approach

The RLI was calculated following an the equal-steps approach (Butchart et al. 2004; Butchart et al. 2007) by assigning ordinal ranks to IUCN Red List categories (see supporting information S2). We replicated the original results of the sampled approach (Baillie et al. 2008).

2.2.3 Data simulations

All analyses were conducted in R v.3.1.2 (R Core Team 2014).

Determining RLI sample size

We revised the methods Baillie et al. (2008) used to match the current RLI protocol (Butchart et al. 2007, see supporting information S2). We tested each taxonomic group independently, by generating subsets of increasing sample size, from 100 to 3,000 species, at increments of 100. For each sample size, we randomly selected species from the group’s species list without replacement, repeating this process 50,000 times and calculating the RLI value for each of these replicates. Using the same threshold as Baillie et al. (2008), we estimated the size of the smallest random subset that accurately detected the trend direction of the full dataset at least 95% of the time. We identified the size of the largest of these subsets across all the inter-assessment periods and taxonomic groups (Baillie et al. 2008).

Minimum sample size to detect trend direction

We calculated trend direction as the direction (positive or negative) of the difference between two RLI values for all known inter-assessment periods (see supporting information S1). We compared the trend direction of each sample with the trend recorded for the entire group over that same period. We repeated this for all 50,000 replicates of each sample size, determining the percentage of simulations that detected the wrong trend direction (i.e. a positive or flat trend when the true RLI was declining).

Minimum sample size to detect changes in slope direction

We also investigated the minimum sample size required to detect changes between RLI slopes, to determine if biodiversity loss is decelerating or accelerating: an approach that has been used to assess progress towards biodiversity targets (Tittensor et al. 2014).

We calculated slope as the difference between two RLI values and compared that value in each sample with the value recorded for the entire group over that same time period (e.g. if slope A between period 1 and 2 is steeper than slope B between period 2 and 3, where slope A > slope B, we considered the detection to be correct if sample slope A > sample slope B but not if sample slope A <= sample slope B).

Effect of inter-assessment length on sample size

We also selectively excluded comprehensive assessments of bird species from our dataset to generate all possible combinations of inter-assessment length (i.e. from 4 up to 28 years in length) and assess the impact of longer inter-assessment periods. Applying the same sampled approach as described in 2.3.2 to simulated inter-assessment lengths (see Supporting Information S3), we compared the percentage of simulations (out of 50,000 replicates) that detected the wrong trend direction for each inter-assessment length.

Representation of taxonomy, biogeography and system in minimum sample sizes

We tested our samples in terms of their representativeness of different systems (marine, freshwater and marine), higher taxonomy (orders or families) and biogeography realms (Palearctic, Neotropical, etc) for birds, mammals, amphibians, corals and cycads (see supporting information S6). For each of these groups, we determined across incremental sample sizes (100 to 1000, in increments of 100), how many simulations differed from the known proportions of relevant attributes using a Pearson's Chi-squared Test (with p<=0.05).

2.3 Results

The minimum sample size that correctly represented the RLI trend direction in at least 95% of the simulated samples was ≤ 200 species for corals (10-years inter-assessment period), cycads (11 years), mammals (12 years) and amphibians (24 years, Figure 2.1 A-D), ≤ 400 species for two of the six-year inter-assessment periods for birds and 2700 species for the other four 4-year inter-assessment periods for birds (Figure 2.1 E-J).

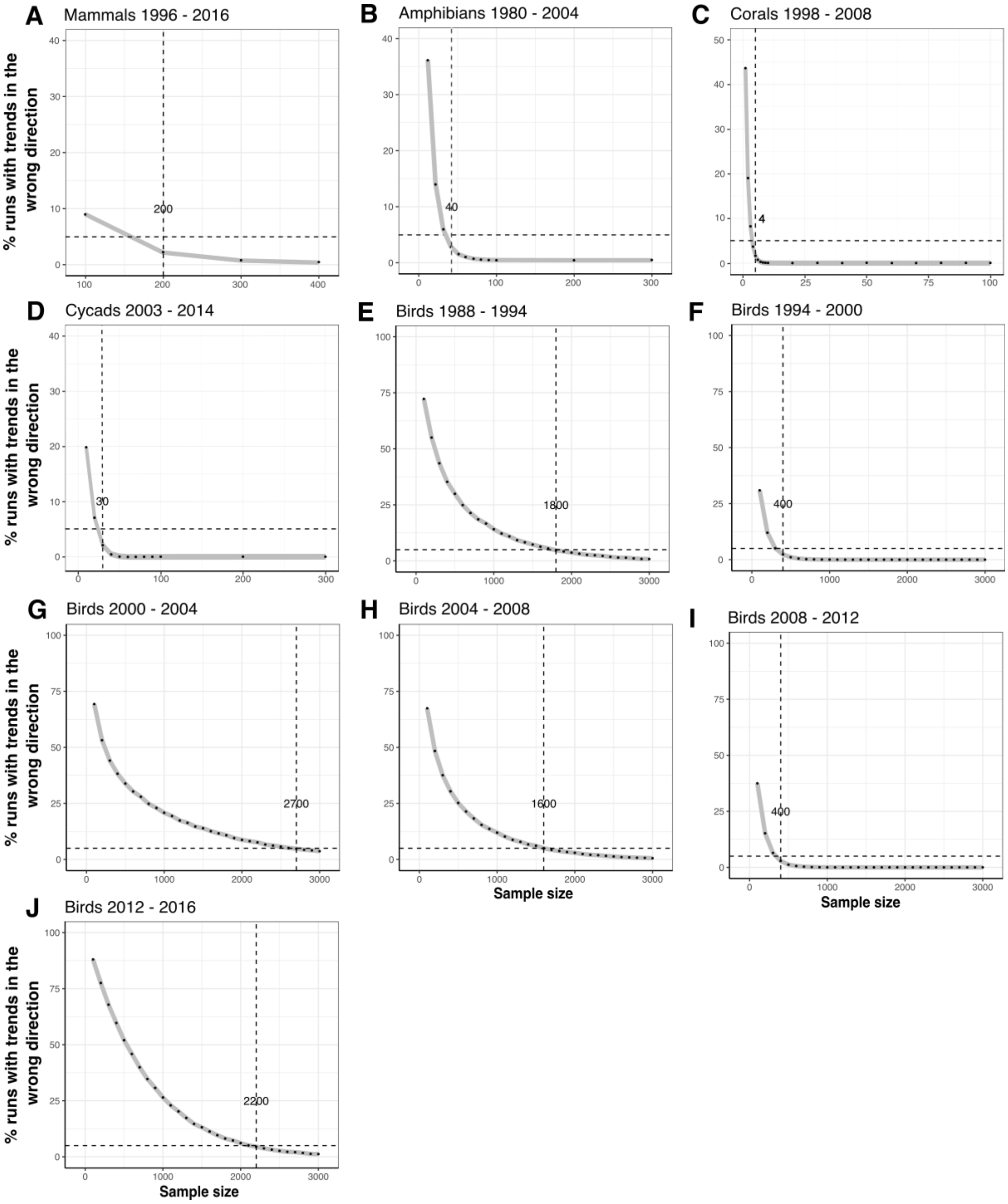

Figure 2.1 - Effect of a sampled approach on the accuracy of detecting RLI trend direction in mammals (A), amphibians (B), reef building corals (C), cycads (D) and birds (E- J), measured as the percentage of the 50,000 replicates that detected a wrong (positive or flat) trend when compared with the complete set of species in that group, which had a negative trend. Horizontal dashed line indicates the threshold for 5%, probability in detecting the wrong direction of the trend (desired 95% accuracy). Vertical dashed line indicates the sample size at which that threshold was met.

Considering birds only, the minimum sample size required to correctly detect changes between slopes (in at least 95% of the subsets) was 11,000 non-DD species (Figure

2.2B), for the period 2000-2004 vs 2004-2008, but 900 non-DD species sufficed for all other inter-assessment periods (Figure 2.2A). For simulations of consecutive slopes with 10 years or longer, a sample of 8900 non-DD was needed (Figure 2.2B).

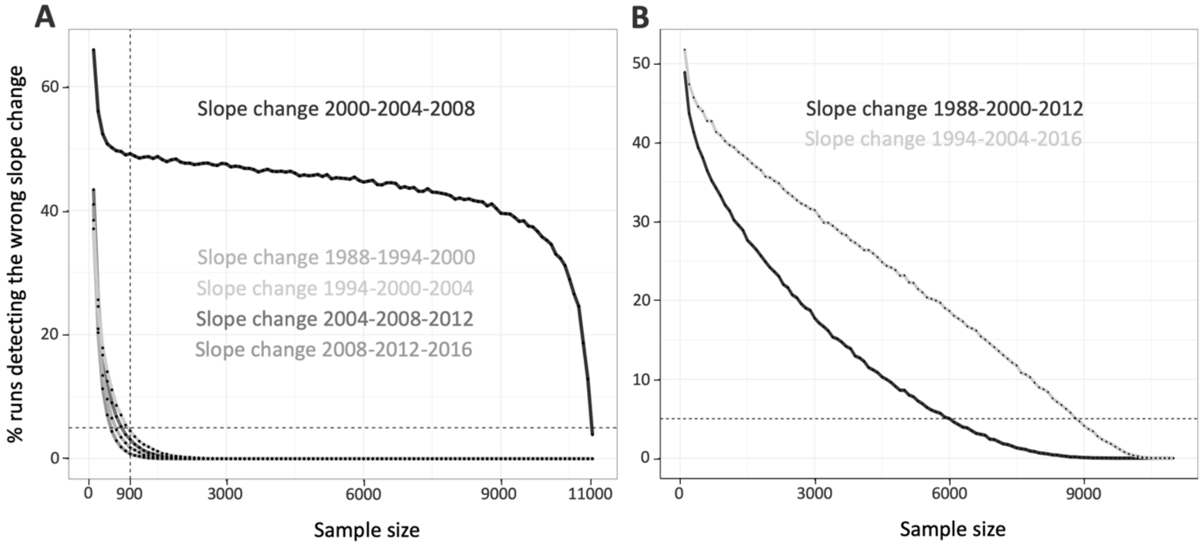

Figure 2.2 - Effect of sample size on the accuracy of slope change detection measured as the percentage of 50,000 replicates. Horizontal dashed line indicates the threshold for 5%, probability in detecting the wrong direction of the trend (desired 95% accuracy), vertical dashed line indicates the sample size at which this threshold was met for most known slopes. Analysis of all available consecutive slopes of birds as they were comprehensively assessed (a) analysis of consecutive slopes of birds with inter-assessment periods of 10 years or longer (b).

When measuring the effect of interassessment length, we found that for periods of ten years or longer, the minimum sample size required to correctly detect trend direction in all species groups was 400 non-DD species, although 200 non-DD species sufficed for all nonavian taxa (Figure 2.3). We also found these sample sizes to accurately reflected attributes regarding biogeographic realm, ecosystem types and taxonomy (Figure 2.4 and Supporting Information S5).

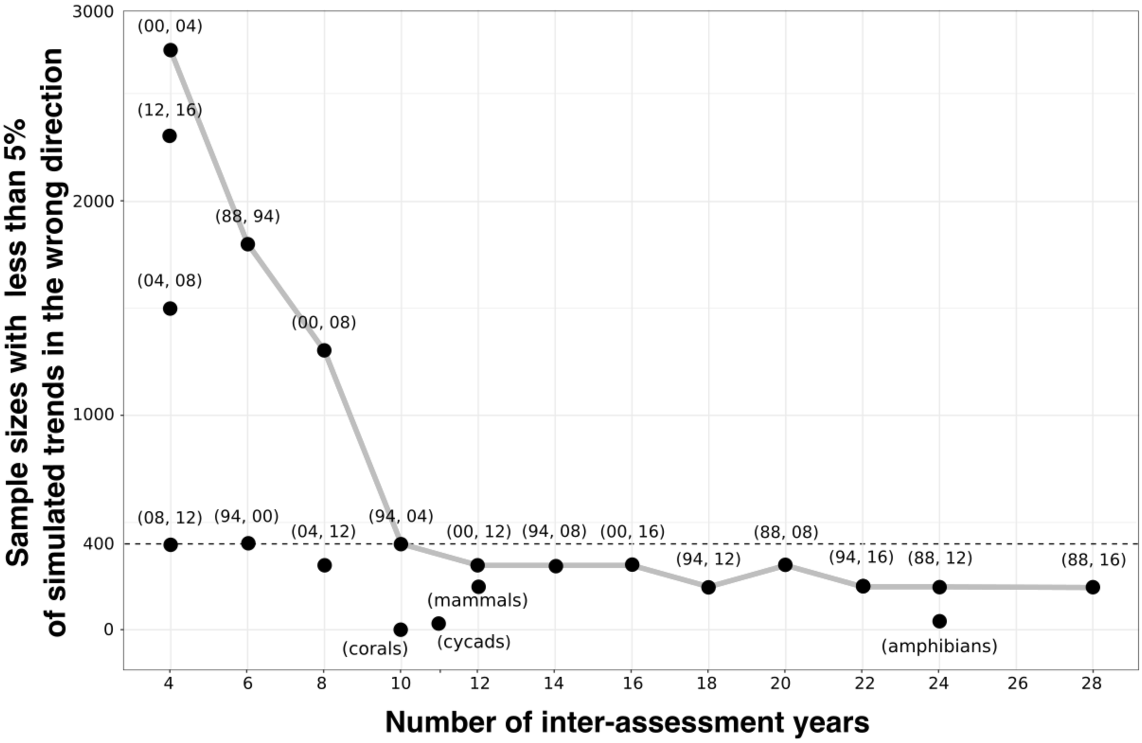

Figure 2.3 - Effect of sample size on the accuracy of trend detection (less than 5% runs in the wrong direction) for all possible combinations of inter-assessment lengths of subsequent year in birds; and the existing RLIs of amphibians , corals, cycads and mammals with less than 5% of runs in the wrong direction are also represented.

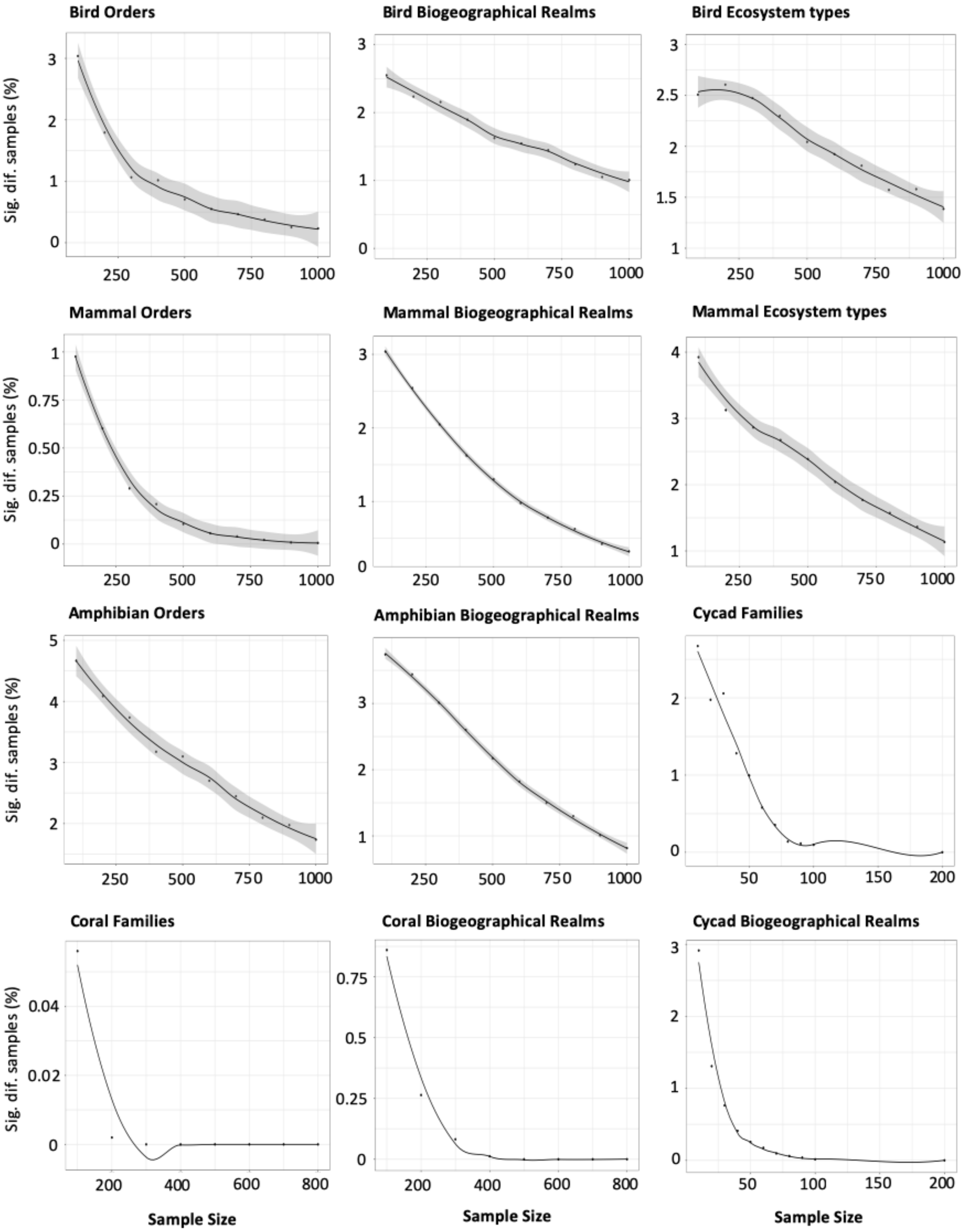

Figure 2.4 - Analysis of different attributes (taxonomy, biogeographical realm and ecosystem types) in increasing sample sizes. Measured as the percentage of samples that were significant different (p<=0.05) to the known proportions of these attributes in Birds, Mammals, Amphibians, Corals and Cycads.

2.4 Discussion

Ten years after the inception of a sampled approach to the Red List Index, we set out to investigate whether the published methodology remains fit for purpose in the context of currency policy targets. We found that the minimum sample size required to implement a sampled RLI is highly depended on the duration of the assessment period and the aim of the test.

Using the current protocol (Baillie et al. 2008), we now estimate that a sample of 2700 species is needed to detect the correct trend direction over time intervals that may be as short as 4 years between assessments (Figure 2.1). However, a sample of 400 species was sufficient for all taxonomic groups when reassessing them every 10 years (Figure 2.3), or just 200 species for non-avian groups.

From a policy perspective, the correct direction of trend provides limited information, given efforts to slow and ‘bend’ the curve of biodiversity loss (Mace et al. 2018). Correctly detecting changes in Red List Index trends for birds (the only group with data available for this test) requires much larger samples of at least 900 species for most time periods and 11,000 species in one instance (Figure 2.2).

Sample size is a crucial issue because red listing can be technically challenging and requires considerable time and resources (Rondinini et al. 2014; Juffe-Bignoli et al. 2016). Our results provide the most robust estimates to date of the numbers of assessments required to provide robust RLIs when using a sampled approach.

Specifically, we observed that when considering an inter-assessment period of 10 years or more, the sample size required to detect the correct direction of trend in at least 95% of simulations was 400 non-DD species (Figure 2.3), instead of the 2700 species needed for 4-year periods (Figure 2.1). Although birds have been reassessed at 4-year intervals since 2000 (and 6-year intervals prior to then), reassessments of cycads and mammals were completed after 11 and 12 years respectively, despite the fact that Red List assessments become out of date after 10 years (IUCN 2012). The original assessment for amphibians in 2004 was accompanied with a retrospective (‘backcast’) assessment for 1980 (24 years), and similarly the assessment for corals in 2008 was accompanied with a retrospective assessment for 1998 (10 years). Repeat assessments of mammals are due in 2020 (12 years after the previous assessment) and of amphibians in 2020 or later (at least 16 years after the previous assessment). Clearly, reassessing large numbers of species on a regular and timely basis poses considerable challenges.

For a 10-year interval, the sample size of 400 species required for detecting the correct direction of trend for birds was double that required for other groups (Figure 2.3). This is because birds are deteriorating in status less rapidly than other groups with known trends, and therefore larger sample sizes are needed to correctly detect shallower RLI slopes.

Similarly, while 900 species were required to detect correctly the changes in slope for most inter-assessment periods for birds (Figure 2.2A), RLI trends across 2000-2004 and 2004-2008. were so similar in value and shallow in slope, that it is necessary to include almost all species in a sample in order to detect the change in slope (Figure 2.2B). We note that 900 species corresponds to the originally recommended sample size by Baillie et al. (2008). The required sample size is likely to be smaller for taxonomic groups that are declining more rapidly, but we were only able to apply this test to bird data as this is the only group for which we have three or more assessments.

Unfortunately, for many species, data on population status and even occurrence data are not gathered regularly to measure changes in red list category within short time frames (Rondinini et al. 2014; Tapley et al. 2018). Notable exceptions are those where we can measure extent of habitat loss from remote-sensed data (e.g. forest dependent species; Buchanan et al. 2008), which can then be incorporated into IUCN Red List assessments through subsequent inference of population reductions. However, equivalent approaches to facilitate Red List assessment updates have not been created for other species groups (e.g. desert or cave dwelling species; Durant et al. 2012; Mammola et al. 2018) or other threats (e.g. wildlife trade, pollution). This entails that even if small samples were reassessed frequently, most groups would likely display a flat trend, not because their extinction risk has not changed, but because the underlying data that informs category change is not being captured timely enough (within 4 to 6 years).

It is therefore clear that the question being addressed and the inter-assessment period considered will define the sample size required for RLIs. With limited conservation resources and over a 10-year reassessment period (now true for most SRLI groups, such as the Odonata; Clausnitzer et al. 2009) a sample of 400 non-DD species could present a feasible reassessment goal, whereas for groups defining a baseline 200 species should be prioritised, expanded to 900 species where possible. We also note that sample sizes of 400 species accurately reflected attributes such as occurrence in terrestrial, marine and freshwater ecosystems, in different biogeographic realms and higher taxa, like order and family (supporting information S6).

Our results have immediate bearing on those taxonomic groups for which sampled assessments have already been undertaken, particularly those assessments with baselines older than 10 years (e.g. Odonata; Clausnitzer et al. 2009). Therefore, priority could be given to reassessing a smaller subset of 400 non-DD species. This could free up vital resources to further broaden the taxonomic scope of sampled Red List assessments by including additional taxa, or to prioritise reassessments of highly threatened species, which require regular updates to monitor the effectiveness of conservation interventions or to determine if these species are further deteriorating in status.

When designing sampled assessments for a new taxonomic group, it is unknown what proportion of species are DD. This proportion varies considerably between groups, from less than 1% DD in pteridophytes and birds (Brummitt et al. 2015; BirdLife International 2018) to 35% in Odonata (Clausnitzer et al. 2009) and 40% in sharks and rays (IUCN 2019), and it may be even higher in understudied groups such as spiders (Seppälä et al. 2018a, b, c, d). While patterns of data deficiency are of importance to conservation action and research, they introduce uncertainty into assessments of extinction risk of species groups (Bland et al. 2012) and DD species do not contribute to the RLI value (other than confidence intervals; Butchart et al. 2010). Therefore, we believe that any sample size recommendation should be based solely on non-DD species for biodiversity indicator development. Based on our result, we would suggest that groups implementing the protocol should pursue the assessment of a set of random species until 400 non-DD species are found (or 900 non-DD species) if aiming to detect change in trends (although this may pose practical challenges, as gathering data may not proceed species by species).

As our analysis is solely based on groups with appropriate data currently available, recommended sample size may prove insufficient to detect accurately the trend direction or slopes in untested groups. However, steeper RLI slopes require a smaller sample size to detect their trend accurately. Therefore, errors are less likely in species groups that are declining more steeply (Figure 2.3). Furthermore for groups previously assessed, reassessments are aided by the fact that the previous baseline assessment has already identified DD species within the sample, thereby creating a pool of non-DD species from which to sub-sample.

We found that the sampled approach to the RLI remains a useful tool as part of efforts to monitor global biodiversity targets (particularly CBD target 12) at a global scale, and a ten-year reassessment interval could accurately and continuously inform on biodiversity trends while minimising resource expenditure.

However, sample size should be carefully balanced against shorter political time-scales or species with quickly deteriorating status. This might require more frequent assessments (in turn requiring larger samples) or regular assessments of target species (those with highest extinction risk). As well as balancing resource and data availability, this might ideally allow for the comprehensive assessments for specific species groups (such as birds) or only feasibility support a smaller, yet still informative, sample size of 200 non-DD sp.

It is vital to effectively determine if global species conservation targets have been met, and to allocate resources where they would be most effective. Despite their scientific importance and the fact that they are necessary to achieve international biodiversity and development goals, Red Listing efforts are insufficiently resourced (Rondinini et al. 2014; Bland et al. 2015; Goettsch et al. 2015; Juffe-Bignoli et al. 2016; Bachman et al. 2018). The IUCN Red List should be treated as a ‘global public good’, and resourced appropriately as a cost-effective and crucial tool to tackle biodiversity loss (Stuart et al. 2010).

Initiating sampled reassessments now, is paramount to provide best-available information for the renegotiation of biodiversity targets post-2020; additionally, new sampled assessments are required to improve our ability to adequately represent and detect biodiversity trends by 2030, coinciding with the year of a revision of the UN

Strategic Plan for Biodiversity. In conclusion, sampled assessments can be an important complement to comprehensive assessments and have a meaningful contribution to understand global extinction patterns. Still, there is a trade-off between what sample size can be feasibly implemented and the information the resulting indices can provide.

2.5. Acknowledgements

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2S. Supplementary Materials

2S.1. Data

We compiled the Red List categories of all taxonomical groups that have been comprehensively assessed, meaning taxonomic groups where the extinction risk of all its species have been assessed under the IUCN Red List criteria, and re-assessed at least once. Which compromised birds, corals, cycads and mammals. This dataset encompasses the most recent assessments, hence including retrospective updates (Butchart et al., 2007, 2010; Hoffmann et al., 2011), identifying genuine changes in species extinction risk category over time (i.e. a genuine improvement or deterioration in Red List category). Category changes owing to improved knowledge, revised taxonomy or other non-genuine changes were excluded (see Butchart et al., 2007, 2010).

The inter-assessment period differs greatly among specific groups, as amphibians where assessed in 1980 and 2004, birds (1988, 1994, 2000, 2004, 2008, 2012, 2016), corals (1996, 2008), cycads (2003, 2014) and mammals (1996, 2008).

All analyses were carried out in R v.3.1.2 (R Core team, 2014), using the R packages “dplyr”, “plyr”, “data.table", “boot”, "doParallel", “foreach”, “plotrix”, “directlabels” and “ggplot2”.

2S.2. The RLI formula and its sample approach

In the equal-steps approach the species classified as Extinct (including Extinct in the Wild and Possibly Extinct) are assigned a weight equal to 5, Critically Endangered =4, Endangered =3, Vulnerable =2, Near Threatened =1 and Least Concern =0 (Butchart et al., 2004, 2007).

The RLI is calculated as:

$$RLI_t = 1 - \frac{\sum_s W_c(t,s)}{W_{Ex} * N}$$

where $\sum_s Wc(t,s)$ is the sum of the weights (*Wc*), of all non-DD Red List categories across all species *s* in a given group in a given year *t*; WEx is the category weight for Extinct (weighted as 5) and *N* is the number of non-DD species in the group (Butchart et al., 2007). In short, the RLI calculates the extinction risk of a group relative to the worst-case scenario where all species in that group would be Extinct (W_{EX}).

The replication of the original results (Baillie et al. 2008) was made with the same dataset for birds and amphibians used by the authors and by applying the same methods, which importantly includes Extinct species from the baseline, the RLI of year one of 1988.

We then updated the methods used by applying the same protocol that is currently used for calculating RLIs to our sampled approach, which excludes Extinct species from the baseline year, as these tend to be much better documented in some groups.

The detection goal of the sampled approach to the RLI was also revised to make it more relevant to the current policy context, where rather than falsely showing a positive index slope when the known trend is negative (Baillie et al., 2008), we calculated the sample size required to detect if species conservation status has been improved and sustained, by detecting slopes in a sampled approached that falsely detected a positive or neutral trend.

2S.3. Effects of inter-assessment length

The current sampled RLI protocol has thus far only analysed the slope between two consecutive comprehensive assessments. We applied this same process to all available groups (see Supplementary Information S1), also using this process to all possible inter-assessment lengths that could be generated by selectively bypassing subsequent comprehensive assessments of bird species (Table S2.1). Allowing us to analyse what was the percentage of simulations (out of the 50,000 subsets) that detected the wrong trend direction for each possible inter-assessment length (Figure S2.1).

Table S2.1 - Possible combinations all possible inter-assessment lengths of subsequent years of bird species. The first row and first column display all the years when the comprehensive assessments were published, length in years between parentheses.

Assessment years	1994	2000	2004	2008	2012	2016
1988	1988-1994 (6 years)	1988-2000 (12 years)	1988-2004 (16 years)	1988-2008 (20 years)	1988-2012 (24 years)	1988-2016 (28 years)
1994	-	1994-2000 (6 years)	1994-2004 (10 years)	1994-2008 (14 years)	1994-2012 (18 years)	1994-2016 (22 years)
2000	-	-	2000-2004 (4 years)	2000-2008 (8 years)	2000-2012 (12 years)	2000-2016 (16 years)
2004	-	-	-	2004-2008 (4 years)	2004-2012 (8 years)	2004-2016 (12 years)
2008	-	-	-	-	2008-2012 (4 years)	2008-2016 (8 years)
2012	-	-	-	-	-	2012-2016 (4 years)

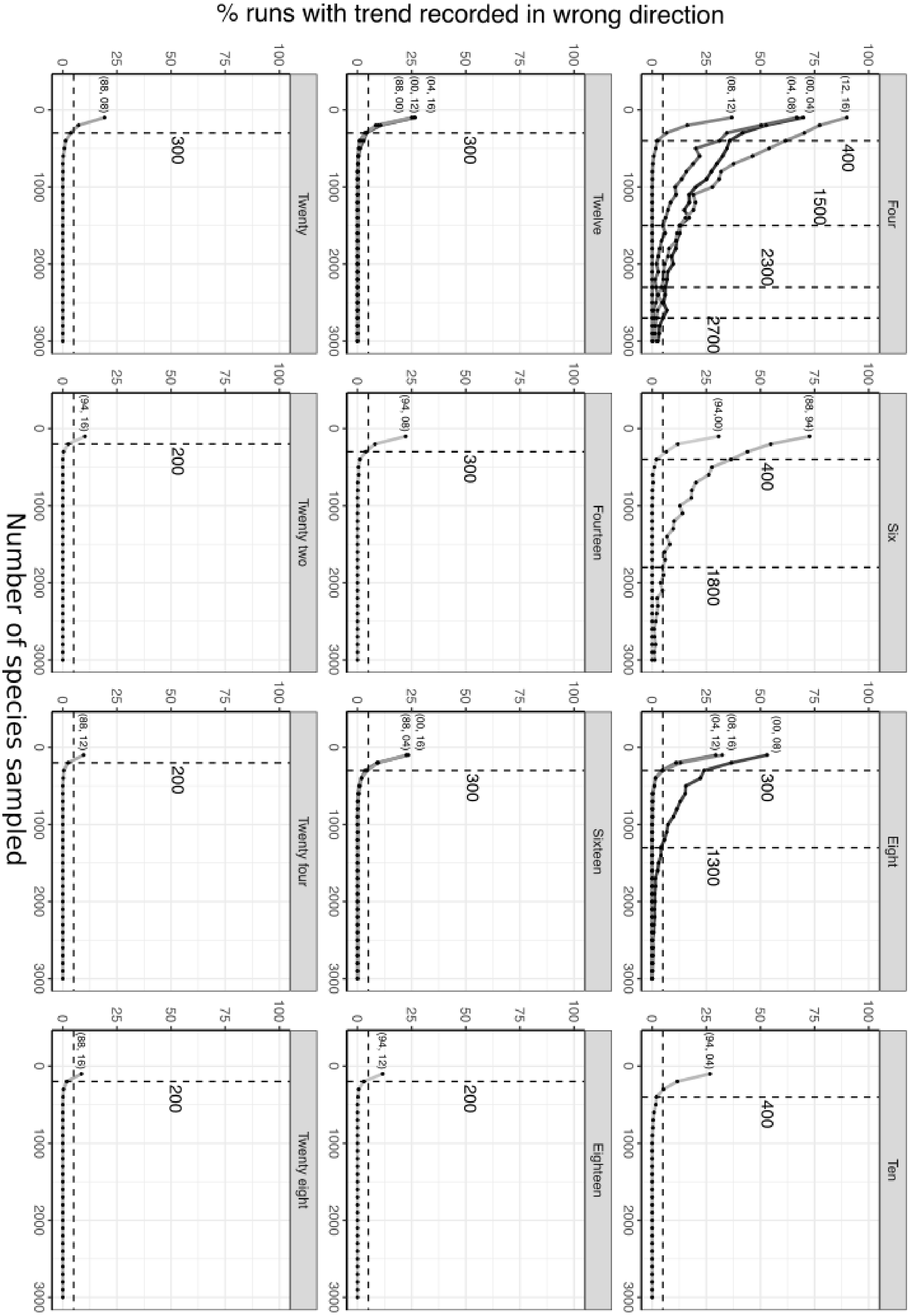

Figure S2.1 - Effect of sample size on the accuracy of trend detection for all possible combinations of inter-assessment lengths of subsequent years, measured as the percentage of replicates that detected the wrong trend direction. Each graphic depicting analysis of the same inter-assessment length, indicated at the top of each individual graphic. Horizontal dashed line indicating the threshold for 5% probability in detecting the wrong direction of the trend (95%accuracy), vertical dashed line indicating the lowest number species that meets this threshold for each of the analysed periods (which are indicated between brackets).

2S.4. Replicating and updating the sampled RLI

The original sampled RLI protocol, included any non-data deficient species into the baseline calculation, including Extinct species (Baillie et al., 2008). However, current guidelines recommend that species that have been assessed as extinct in year 1 (baseline), should not be included in the slope calculation. When we exclude extinct species from the baseline calculation, it impacts the RLI values, the inter-assessment slope and consequently the sample needed to detect a trend direction (Figure S2.2 and Figure S2.3).

The sampled RLI protocol was originally established to contribute towards monitoring decision VI/26 of the CBD (Baillie et al., 2008), which was set “to achieve a significant reduction of the current rate of biodiversity loss at the global, regional and national level as a contribution to poverty alleviation and to the benefit of all life on earth by 2010” (Balmford et al., 2005). Therefore a fair interpretation of this was to measure “extent of biodiversity loss over time” by “testing the probability of a sampled RLI falsely showing an apparent reduction in the rate of biodiversity loss (i.e., a positive slope to the index: see Butchart et al., 2007) when the known trend is negative." (Baillie et al., 2008).

However, the current 2020 target is aimed at measuring if "the extinction of known threatened species has been prevented and their conservation status, particularly of those most in decline, has been improved and sustained” (target 12 of the Aichi Biodiversity Targets).

Revising the sampled RLI to address this target, we considered that besides detecting improvements (positive trends), "sustained" would correspond to detecting a flat trend (representing no change in sampled RLI value) which is therefore important to detect as a wrong trend in sampled RLI simulations, which may sound like a minor detail, but that significantly impacts sample size (Figure S2.2 and Figure S2.3).

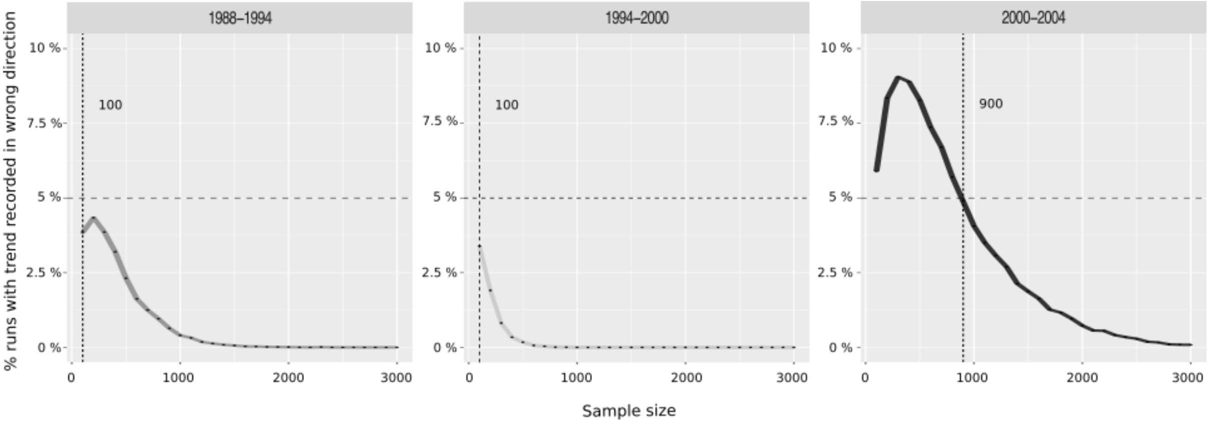

Figure S2.2 - Effect of the sample size in detecting a positive trend direction (trend in wrong direction) between RLI values (using the available bird data in 2007), including Extinct species from the baseline (original sRLI formula), dashed line indicating the threshold for 5% probability in detecting the wrong direction of the trend (95%accuracy).

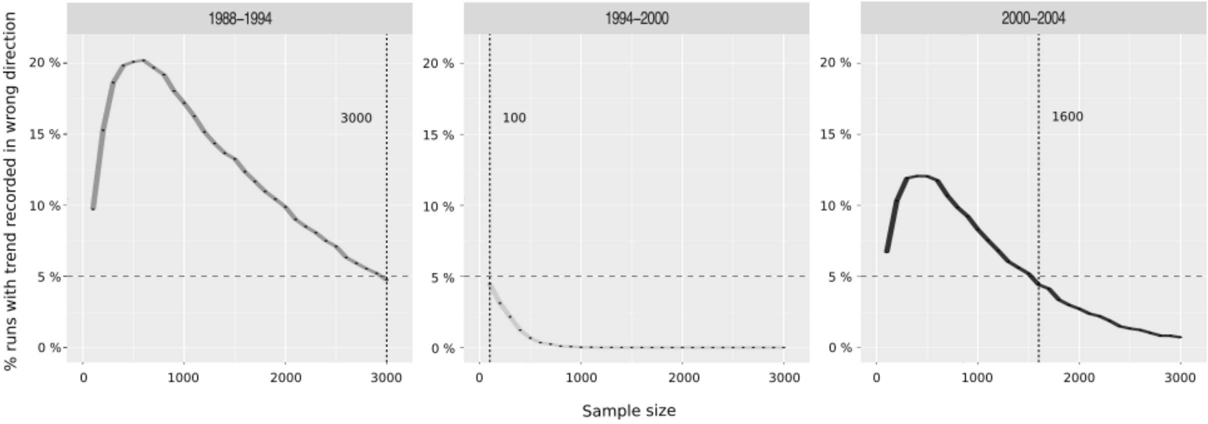

Figure S2.3 - Effect of the sample size in detecting a positive trend direction (trend in wrong direction) between RLI values (using the available bird data in 2007), excluding Extinct species from the baseline (original sRLI formula), dashed line indicating the threshold for 5% probability in detecting the wrong direction of the trend (95%accuracy).

2S.5. Representing subsets

We performed to distinct analysis to test how representative would different sample size subsets be:

Analysis 1:

We tested if the proportion of relevant attributes (taxonomy, biogeographical realms and ecosystem types) was not significantly different in more than 95% of simulated samples sizes of all available groups (Figure 2.4). Although we note this might not be most appropriate test to detect/incorporate rare cases (e.g. taxonomic orders with one species) and a more comprehensive analysis of attribute representability should be the focus of future research.

For this analysis, the likelihood of a random sample to be representative of Coral orders was not analysed. As the reef building species present in the group's RLI are taxonomically grouped into three very species-unbalanced orders, Alcyonaceae – 1sp., Helioporacea – 1sp., Scleractinia- 825 sp., which entail that the proportion of significantly different samples is very close to 0 even for very small sample sizes (eg.10 sp.). Therefore we analysed the likelihood of a random sample to be representative of Coral families. We followed the same approach for Cycads (which all belong to the same Order Cycadales), and following the same line of reasoning it would be uninformative to analyse Coral Ecosystems Types (as all species where marine), Cycads Ecosystem types (as all species are terrestrials), nor Amphibian Ecosystems types (as almost all species inhabit Fresh water), therefore these analysis where excluded.

Analysis 2:

For the sample size that met the minimum sample thresholds (see methods) for a ten year inter-assessment period, 400 species (Figure 2.3), we replicated the original sampled RLI exploration of subset patterns in birds (Baillie et al., 2008) to allow for our results to be compared with previous findings. We did this by recording how the 95% confidence intervals of 50,000 samples resembled, in terms of threat classes, biogeographic realms, ecosystem types and taxonomic orders, the known proportions of these attribute in all Bird species.

For the analysis of sampled subsets representing threat classes, we analysed all available comprehensive assessments of bird species (1988, 1994, 2000, 2004, 2008, 2012, 2016), clustering species red listed as Extinct, Extinct in the Wild and Possibly Extinct into the eExtinct (EX) class, all species red listed as Critically Endangered, Endangered and Vulnerable into the threatened (th) class, all species red listed as Near Threatened and Least Concern into the non-threatened (nt) class, and Data Deficient (dd) species into a class with that name (Figure S2.4).

For the analysis of biogeographic realms, we used the same bird species dataset analysed in the original sampled RLI and compared the proportion of species found in the: Palearctic, Afrotropical, Indomalayan, Australasia/Oceania, Nearctic or Neotropical realms (Figure S2.5).

For the analysis of subsets according to ecosystem types, we also used the original database and compared the proportion of species inhabiting Terrestrial, Marine or Freshwater habitats (Figure S2.6).

For the analysis of subsets according to Taxonomic Orders, we used the most updated taxonomic database, as recognized by the latest version of the Handbook of the Birds of the World and BirdLife Taxonomic Checklist (v2), and compared the proportion of species found in each of the 35 recognized bird Orders (Figure S2.7).

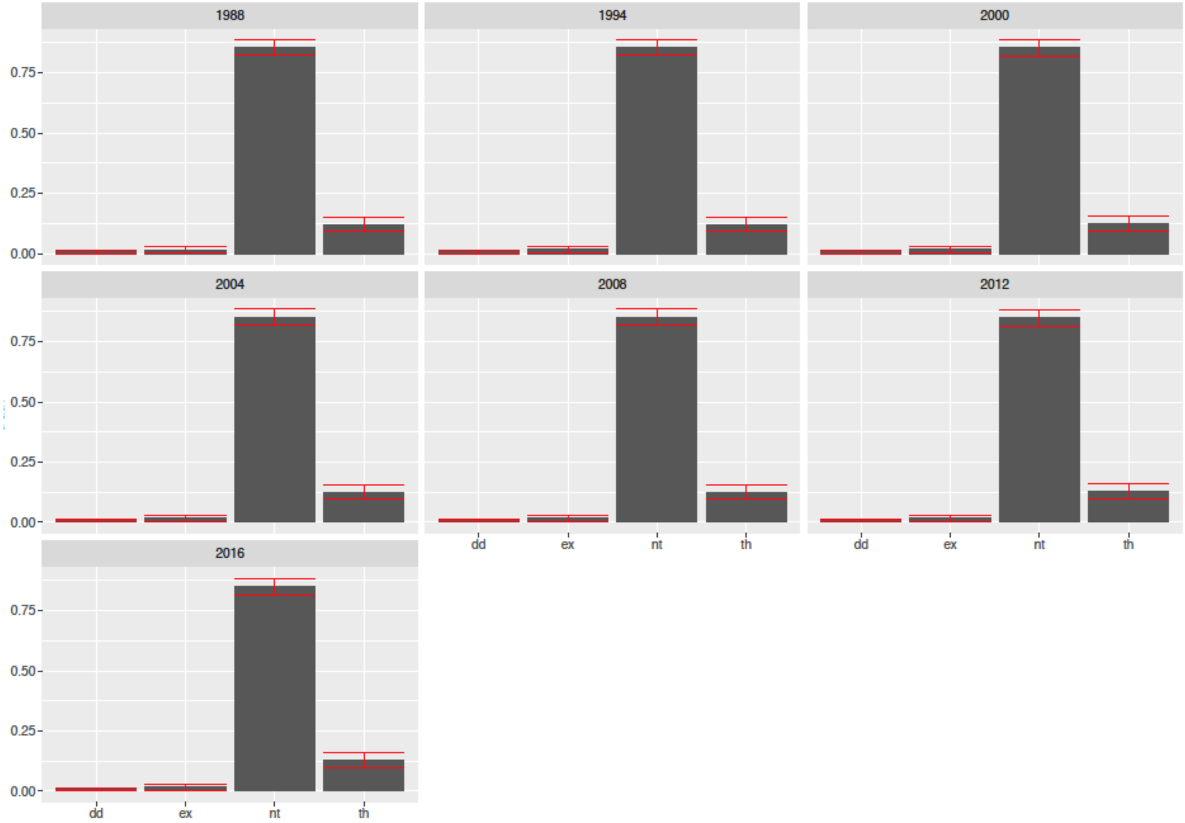

Figure S2.4 - Distribution of threat classes (dd- data deficient; ex – extinct, nt – non-threatened, th - threatened), comparing the 95% confidence intervals of 50,000 samples of 400 Birds (confidence intervals in red) with the truth, all bird species for all available comprehensively assessed years.

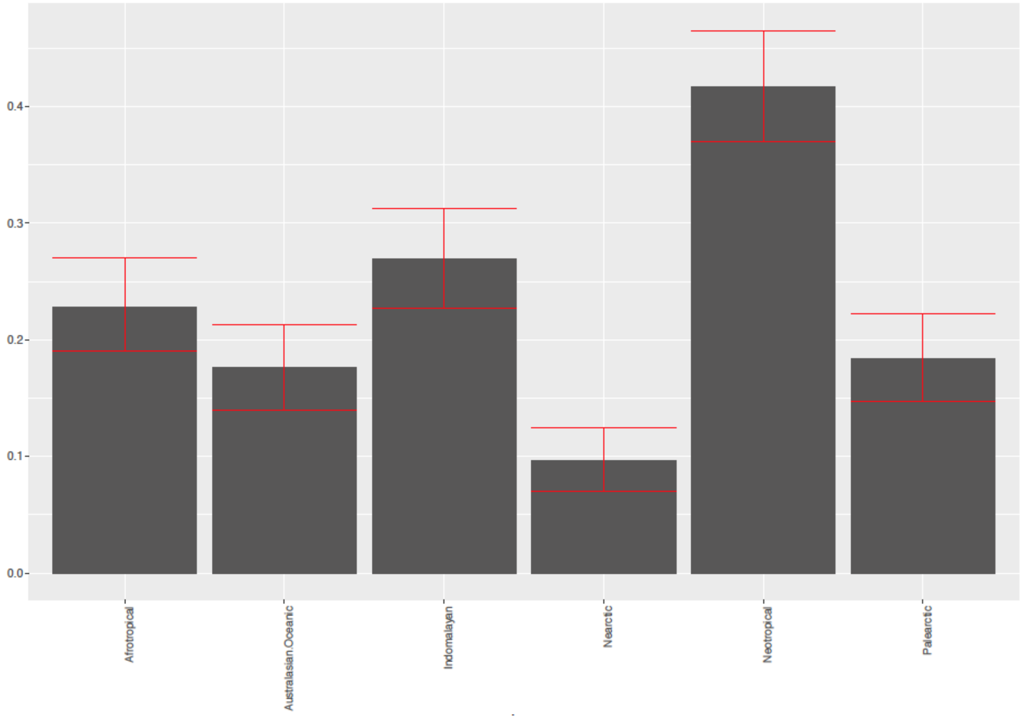

Figure S2.5 - Distribution of biogeographic realms comparing the 95% confidence intervals of 50.000 samples of 400 Birds (in red) with the data available for all bird species at the time of sRLI inception (2007).

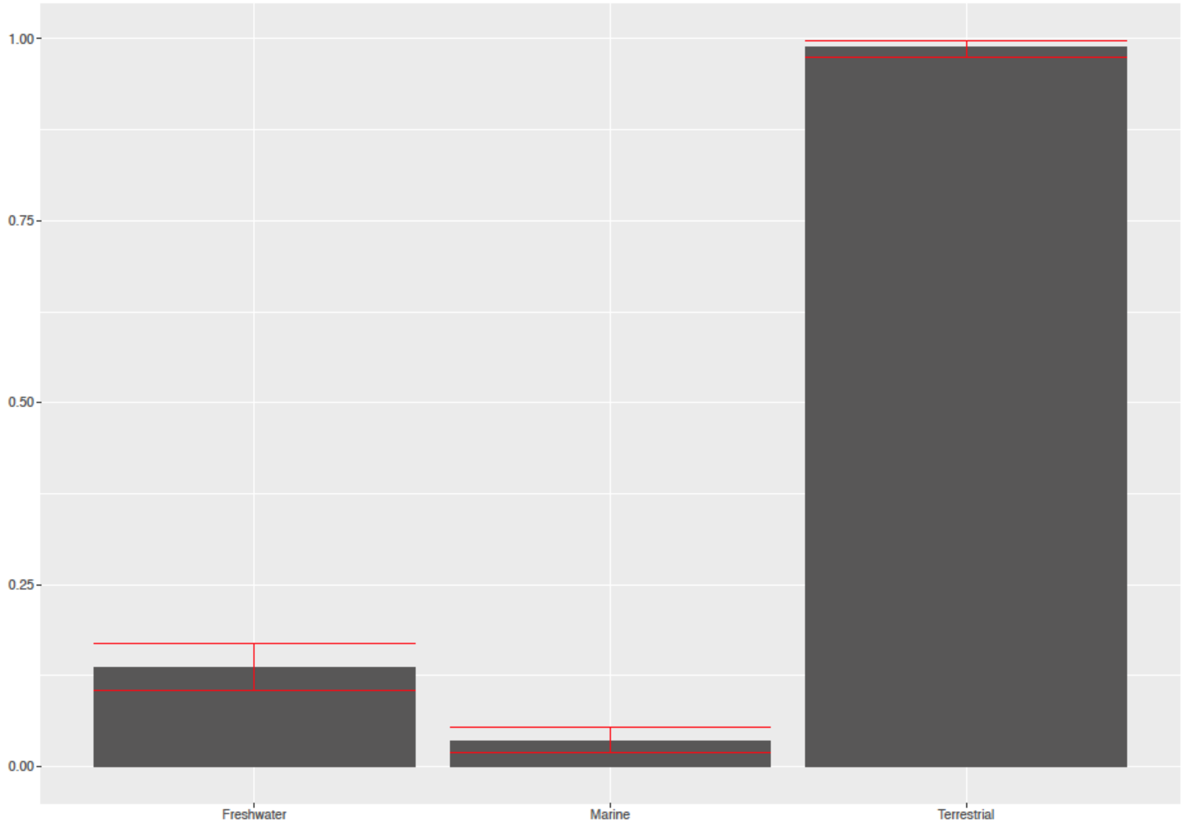

Figure S2.6 - Distribution of ecosystems types comparing the 95% confidence intervals of 50.000 samples of 400 Birds (in red) with the data available for all bird species at the time of sampled RLI inception (2007).

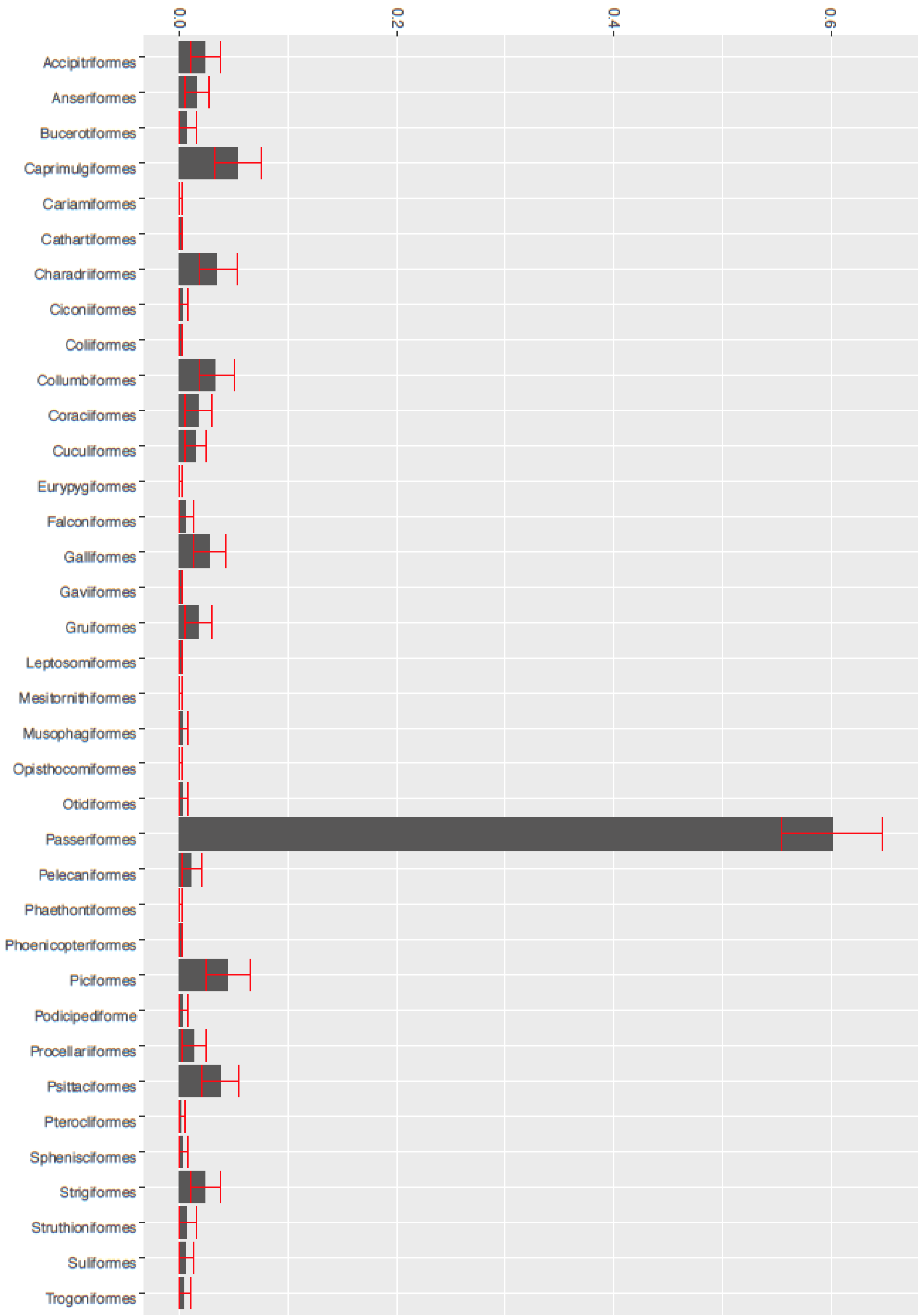

Figure S2.7 - Distribution of taxonomic orders comparing the 95% confidence intervals of 50.000 samples of 400 Birds (in red) with the most updated data for all bird (as per BirdLife international).

2S.6. Code

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```
library(ggplot2)
library(doParallel)
library(plyr)
library(data.table)
library(directlabels)
library(tidyverse)
```

1) Replication and updating of the SRLI

Replicating the original SRLI approach and it’s plots - from Baillie et al. 2008

Leadoff SRLI approach

In order to replicate the leadoff SRLI results (Fig.1 in Baillie et al. 2008) we used the Red List data for Birds, as available then (in 2007)

```
birds<-read.delim("birds_RLIchanges.txt",header=T,as.is=TRUE)

#we remove all data deficient species
birds <- subset(birds, X1988RL_cat != "DD")
birds <- subset(birds, X1994RL_cat != "DD")
birds <- subset(birds, X2000RL_cat != "DD")
birds <- subset(birds, X2004RL_cat != "DD")
```

We implemented the Red List Index, using the standart incremental weights

```
# set up the weights
wtEX<-5
wtPE<-5
wtEW<-5
wtPEW<-5
wtCR<-4
wtEN<-3
wtVU<-2
```

```
wtNT<-1
wtLC<-0
```

Implementing the SRLI formula as provided by the original authors;

```
Baillie_etal_RLI <- function(species_categories88, species_categories) {
 # Get the number of species in each category
 nEX<-sum(species_categories=="EX", na.rm = T)
 nPE<-sum(species_categories=="PE", na.rm = T)
 nEW<-sum(species_categories=="EW", na.rm = T)
 nPEW<-sum(species_categories=="PEW", na.rm = T)
 nCR<-sum(species_categories=="CR", na.rm = T)
 nEN<-sum(species_categories=="EN", na.rm = T)
 nVU<-sum(species_categories=="VU", na.rm = T)
 nNT<-sum(species_categories=="NT", na.rm = T)
 nLC<-sum(species_categories=="LC", na.rm = T)

 # Get the number of species extinct in year one, category
 nEX88<-sum(species_categories88=="EX", na.rm = T)
 nPE88<-sum(species_categories88=="PE", na.rm = T)
 nEW88<-sum(species_categories88=="EW", na.rm = T)
 nPEW88<-sum(species_categories88=="PEW", na.rm = T)
 nCR88<-sum(species_categories88=="CR", na.rm = T)
 nEN88<-sum(species_categories88=="EN", na.rm = T)
 nVU88<-sum(species_categories88=="VU", na.rm = T)
 nNT88<-sum(species_categories88=="NT", na.rm = T)
 nLC88<-sum(species_categories88=="LC", na.rm = T)

 # Multiply these by the respective weights and sum
 Total<-
(wtEX*nEX)+(wtPE*nPE)+(wtEW*nEW)+(wtPEW*nPEW)+(wtCR*nCR)+(wtEN*nEN)+(wtV
U*nVU)+(wtNT*nNT)+(wtLC*nLC)

 # Work out the worse case scenario
 M<-((nEX88+nEW88+nCR88+nEN88+nVU88+nNT88+nLC88)*wtEX)

 # Calculate RLI
```

```
 (RLI<-((M-Total)/M))

 return(RLI)
}
```

We validated our replication, as we obtained the same values for the four comprehensive assessment of birds available at the time

```
RLI1988 <- Baillie_etal_RLI(birds$X1988RL_cat, birds$X1988RL_cat)
RLI1994 <- Baillie_etal_RLI(birds$X1994RL_cat, birds$X1988RL_cat)
RLI2000 <- Baillie_etal_RLI(birds$X2000RL_cat, birds$X1988RL_cat)
RLI2004 <- Baillie_etal_RLI(birds$X2004RL_cat, birds$X1988RL_cat)

Baillie_etal_RLI_values <-
data.frame(RLI1988=RLI1988,RLI1994=RLI1994,RLI2000=RLI2000,RLI2004=RLI2004)
```

We applied the same SRLI protocol to the same approach, in subsets of increasing sample size, from 100 species to a maximum of 3,000 at increments of 100. For each sample size, we randomly selected species from the species list, without replacement, and we repeated this 50,000 times for each sample size

```
sim = 50000
samplist = seq(100, 3000, by=100)
units<-1:length(birds$binomial)
samp <- nrow(birds)

for (samp in samplist) {
 # Obtain a random sample
 results <- foreach (i = 1:sim, .combine="rbind") %do% {
 random.sample<-sample(units,samp)
 random.birds<-birds[random.sample,]

 # Apply the RLI formula to random samples of each available year
 RLI1988 <- Baillie_etal_RLI(random.birds$X1988RL_cat,
random.birds$X1988RL_cat)
 RLI1994 <- Baillie_etal_RLI(random.birds$X1988RL_cat,
```

```
random.birds$X1994RL_cat)
 RLI2000 <- Baillie_etal_RLI(random.birds$X1988RL_cat,
random.birds$X2000RL_cat)
 RLI2004 <- Baillie_etal_RLI(random.birds$X1988RL_cat,
random.birds$X2004RL_cat)

 # store results per sample size for each assessment year
 this_result =
data.frame(RLI1988=RLI1988,RLI1994=RLI1994,RLI2000=RLI2000,RLI2004=RLI20
04)
 results = cbind(this_result)
 }

 # writes it out to a csv files
 res_filename = paste("50000sRLI_Baillie", samp, "sp.csv", sep="")
 write.csv(results, file=res_filename)
}
```

Using a linear model to determine the slope direction to determine the slope direction between the simulated SRLI values

```
get_slope <- function(samp, idx) {
 y = unlist(samp)[idx]
 x = 1:length(y)
 if (var(y, na.rm=T) == 0) {
 return(0)
 } else {
 res <- lm(y~x)$coef[2]
 return(res)
 }
}
```

Considering only the slopes with negative trend as correct, as in:

perc_wrong = sum(trends > 0)/length(trends)*100

We measure the percentage of wrong trends detected

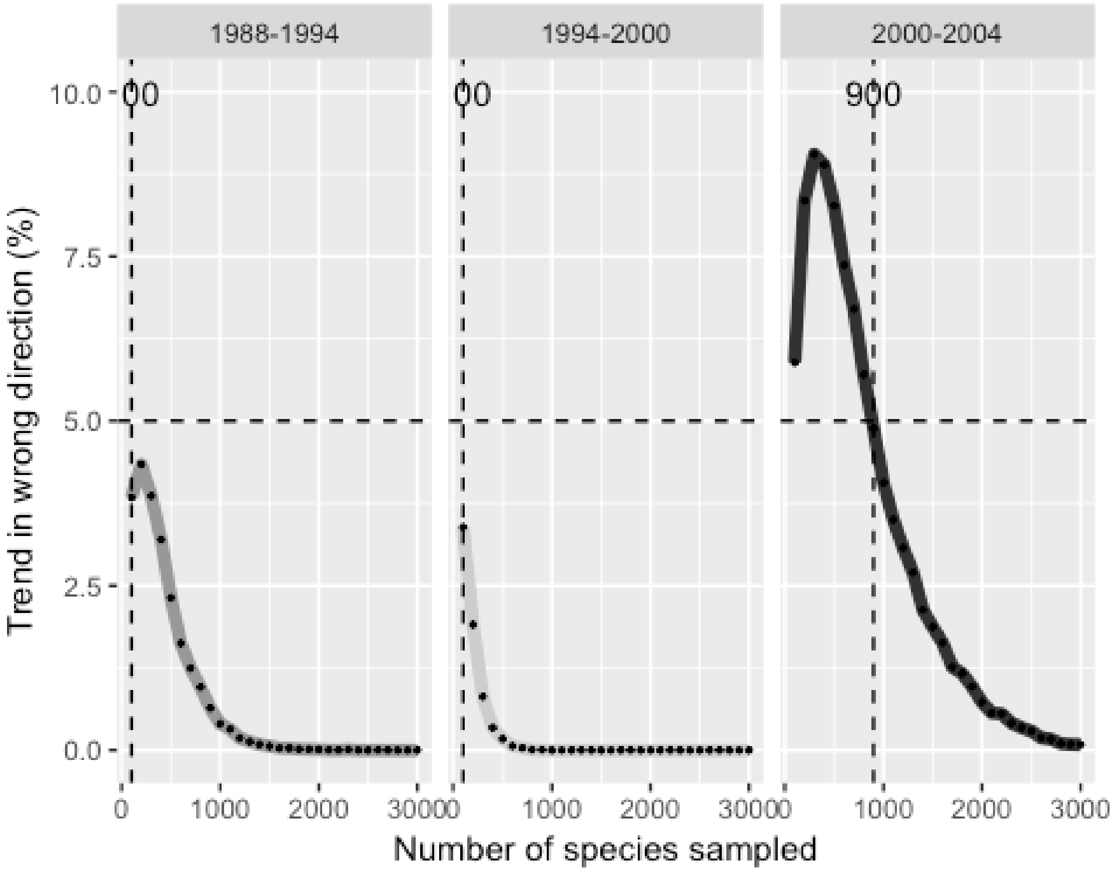
```r
results = list()
i = 1
# Extract metrics from results..
for (samp in samplist) {
 # Get data
 res_filename = paste("50000sRLI_Baillie", samp, "sp.csv", sep="")
 data <- read.csv(res_filename, row.names = 1)
 all_assessments = data[, 1:4]
 resB1 <- apply(all_assessments, 1, get_slope, idx=c(1, 2))
 resB2 <- apply(all_assessments, 1, get_slope, idx=c(2, 3))
 resB3 <- apply(all_assessments, 1, get_slope, idx=c(3, 4))
 res_data = list()
 trend_list = list(resB1, resB2, resB3)
 trend_names = list("(88, 94)", "(94, 00)", "(00, 04)")
 n_assessments = list("Two","Two","Two")

 for (j in 1:3) {
 trends = trend_list[[j]]

 res_data[[j]] = data.frame(
 trend = mean(trends),
 trend_upr = quantile(trends, probs = 0.975),
 trend_lwr = quantile(trends, probs = 0.025),
 perc_wrong = sum(trends > 0)/length(trends)*100,
 label = trend_names[[j]],
 n_ass = n_assessments[[j]],
 samp_size = samp,
 ass_years = paste(trend_names[j])
 )
 }
 res_data = do.call(rbind, res_data)

 results[[i]] = res_data
 i = i + 1
}
```

```
results_df_a = do.call(rbind, results)
write.csv(results_df_a, "Replicating_Baillies_SRLI.csv")
```

Figure S2.2

Ploting our results in a similar fashion to Baillie et al. we can consider our analysis as a validated replication, as we get the same exact results

```
results_df_a <-read.csv("Replicating_Baillies_SRLI.csv")
results_df_a$n_ass_o = factor(results_df_a$ass_years, levels = c("(88,
94)", "(94, 00)", "(00, 04)"), labels = c("1988-1994", "1994-2000",
"2000-2004"))
test_a = ddply(results_df_a, .(n_ass_o), summarize, threshold =
samp_size[min(which(perc_wrong <= 5))])



# Plot line-graphs of % of incorrect slopes...
perc_plot <- ggplot(results_df_a, aes(x=samp_size, y=perc_wrong,
color=ass_years)) +
 geom_line(size=2) +
 geom_point(size=0.5, color="black") +
 geom_hline(yintercept=5, linetype="dashed") +
 ylab("Trend in wrong direction (%)") +
 xlab("Number of species sampled") +
 ylim(0,10) +
 theme(legend.position='none') +
 scale_colour_grey("Replication") +
 facet_grid(~n_ass_o, scales='free') +
 geom_vline(data = test_a, aes(xintercept=threshold), color="black",
linetype="dashed") +
 geom_text(data = test_a, aes(x=threshold, label=threshold, y=10),
color="black")



perc_plot
```



BirdLife's SRLI 2007

Using the RLI formula that is currently used by BirdLife International to produce the official RLI values, and applying it to the bird data available in 2007.

```
updated_RLI <- function (species_categories, ind=NULL, N_EX_YR1,
N_EXTANT_YR1)
{
 if (!is.null(ind)) {
 species_categories = species_categories[ind]
 }
 # Get the number of species in each category
 nEX<-sum(species_categories=="EX" | species_categories=="E", na.rm = T)
 nPE<-sum(species_categories=="CR(PE)" | species_categories=="CR (PE)",
na.rm = T)
 nEW<-sum(species_categories=="EW", na.rm = T)
 nPEW<-sum(species_categories=="CR(PEW)", na.rm = T)
 nCR<-sum(species_categories=="CR", na.rm = T)
```

```
 nEN<-sum(species_categories=="EN", na.rm = T)
 nVU<-sum(species_categories=="VU" | species_categories=="T", na.rm = T)
 nNT<-sum(species_categories=="NT" | species_categories=="LR/nt", na.rm
= T)
 nLC<-sum(species_categories=="LC" | species_categories=="LR/lc" |
species_categories=="LR/cd", na.rm = T)

 #worse case scenario
 New_M <- N_EXTANT_YR1*wtEX

 # Multiply these by the respective weights and sum
 New_T <- (wtEX*((nEX + nPE) -
N_EX_YR1))+(wtEW*nEW)+(wtPEW*nPEW)+(wtCR*nCR)+(wtEN*nEN)+(wtVU*nVU)+(wtN
T*nNT)+(wtLC*nLC)

 # Calculate RLI
 RLI<-((New_M-New_T)/New_M)
 return(RLI)
}
```

When can apply this formula to the sRLI protocol, under the same type of random subsets of increasing sample size

```
sim = 50000
samplist = seq(100, 4000, by=100)

for (samp in samplist) {

 # Loop to calculate RLI values
 results <- foreach (i = 1:sim, .combine="rbind") %do% {
 random.sample<-sample(units,samp)
 random.birds<-birds[random.sample,]

 # Calculate number of species extinct or Potentially Extinct in year 1
 n_expe = sum(random.birds$X1988RL_cat == "EX" |
random.birds$X1988RL_cat == "CR (PE)" | random.birds$X1988RL_cat ==
"CR(PE)")
```

```
 # Number non-extinct, non-PE in year 1
 number_nonex = sum(random.birds$X1988 != "DD") - n_expe

 RLI1988 <- updated_RLI(random.birds$X1988RL_cat, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
 RLI1994 <- updated_RLI(random.birds$X1994RL_cat, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
 RLI2000 <- updated_RLI(random.birds$X2000RL_cat, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
 RLI2004 <- updated_RLI(random.birds$X2004RL_cat, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)

 # store results for each year of assessment for this sample
 this_result =
data.frame(RLI1988=RLI1988,RLI1994=RLI1994,RLI2000=RLI2000,RLI2004=RLI20
04)

 # Add to results
 results = cbind(this_result)
 }

 # writes out to a file
 res_filename = paste("50000sRLI_Birlife", samp, "sp.csv", sep="")
 write.csv(results, file=res_filename)
}
```

Using the same linear model (get_slope), while also considering only the slopes with negative trend as correct

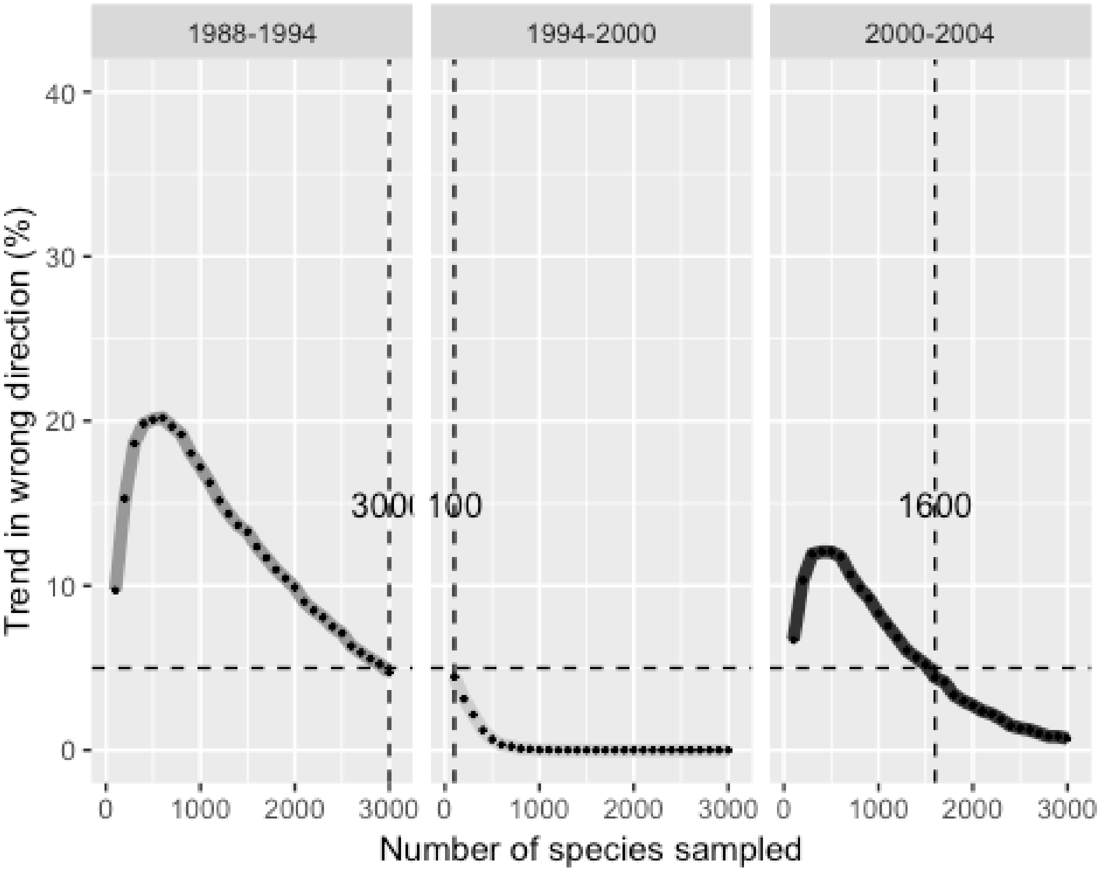
```
results = list()
i = 1
# Extract metrics from results..
for (samp in samplist) {
 # Get data
 res_filename = paste("50000sRLI_Birlife", samp, "sp.csv", sep="")
 data <- read.csv(res_filename, row.names = 1)
```

```
 all_assessments = data[, 1:4]

 resB1 <- apply(all_assessments, 1, get_slope, idx=c(1, 2))
 resB2 <- apply(all_assessments, 1, get_slope, idx=c(2, 3))
 resB3 <- apply(all_assessments, 1, get_slope, idx=c(3, 4))

 res_data = list()
 trend_list = list(resB1, resB2, resB3)
 trend_names = list("(88, 94)", "(94, 00)", "(00, 04)")
 n_assessments = list("Two","Two","Two")

 for (j in 1:3) {
 trends = trend_list[[j]]

 res_data[[j]] = data.frame(
 trend = mean(trends),
 trend_upr = quantile(trends, probs = 0.975),
 trend_lwr = quantile(trends, probs = 0.025),
 perc_wrong = sum(trends > 0)/length(trends)*100,
 label = trend_names[[j]],
 n_ass = n_assessments[[j]],
 samp_size = samp,
 ass_years = paste(trend_names[j])
 )
 }
 res_data = do.call(rbind, res_data)

 results[[i]] = res_data
 i = i + 1
}
results_df_b = do.call(rbind, results)
write.csv(results_df_b, "BirdLife_RLI_formula.csv")
```

Figure S2.3

When plotting these results we can visualize the impact of these two RLI formulas on the same data set.

```
# Plot line-graphs of % of incorrect slopes...
results_df_b <-read.csv("BirdLife_RLI_formula.csv")
results_df_b$n_ass_o = factor(results_df_a$ass_years, levels = c("(88,
94)", "(94, 00)", "(00, 04)"), labels = c("1988-1994", "1994-2000",
"2000-2004"))
test_b = ddply(results_df_b, .(n_ass_o), summarize, threshold =
samp_size[min(which(perc_wrong <= 5))])


# Plot line-graphs of % of incorrect slopes...
perc_plot <- ggplot(results_df_b, aes(x=samp_size, y=perc_wrong,
color=ass_years)) +
 geom_line(size=2) +
 geom_point(size=0.5, color="black") +
 geom_hline(yintercept=5, linetype="dashed") +
 ylab("Trend in wrong direction (%)") +
 xlab("Number of species sampled") +
 xlim(0,3100) +
 ylim(0,40) +
 theme(legend.position='none') +
 scale_colour_grey("Replication") +
 facet_grid(~n_ass_o, scales='free') +
 geom_vline(data = test_b, aes(xintercept=threshold), color="black",
linetype="dashed") +
 geom_text(data = test_b, aes(x=threshold, label=threshold, y=15),
color="black")


perc_plot
```



Exploratory analylis of the plots: When using the same data to compare the differences between "Baillie's RLI" and the "Bird Life's RLI" approaches, it is clear that these approaches affects the minimum sample size of the SRLI significantly (as the new recomended sample size would be 2800 instead of 900), and here is why:

The formula used in both case is: RLI = (M-T)/M
Where as explained in the methods:
- M is the maximum threat score of year 1 - T is the total number of sp. in each category multiplied by the assigned weight to that category.

The difference is that "Baillie's M" includes the species that where Extinct or Potentially Extinct in Year 1:
-> (nEXy1+nPEy1) x wtEX Whereas "Bird Life's M" does not (which means that Baillie_M > Birdlife_M)

Once "Baillie's T" also includes (wtEX x nEX)+(wtPE x nPE), these two values cancel out in year 1. However, for the following years the value of (nEXy1 +

nEXy1) < (nEXyX+nPEyX). Hence the major difference in the first graph (1988-1994), which uses year 1.

Updated SRLI

Considering that not only positive trends of simulated SRLI should be considered wrong, but that by following the 2020 targets, any sample that detects no change in value when a decline has been recorded, should also be considered wrong.

We updated the SRLI correct trend detection to reflect that: perc_wrong = sum(trends >= 0)/length(trends)*100

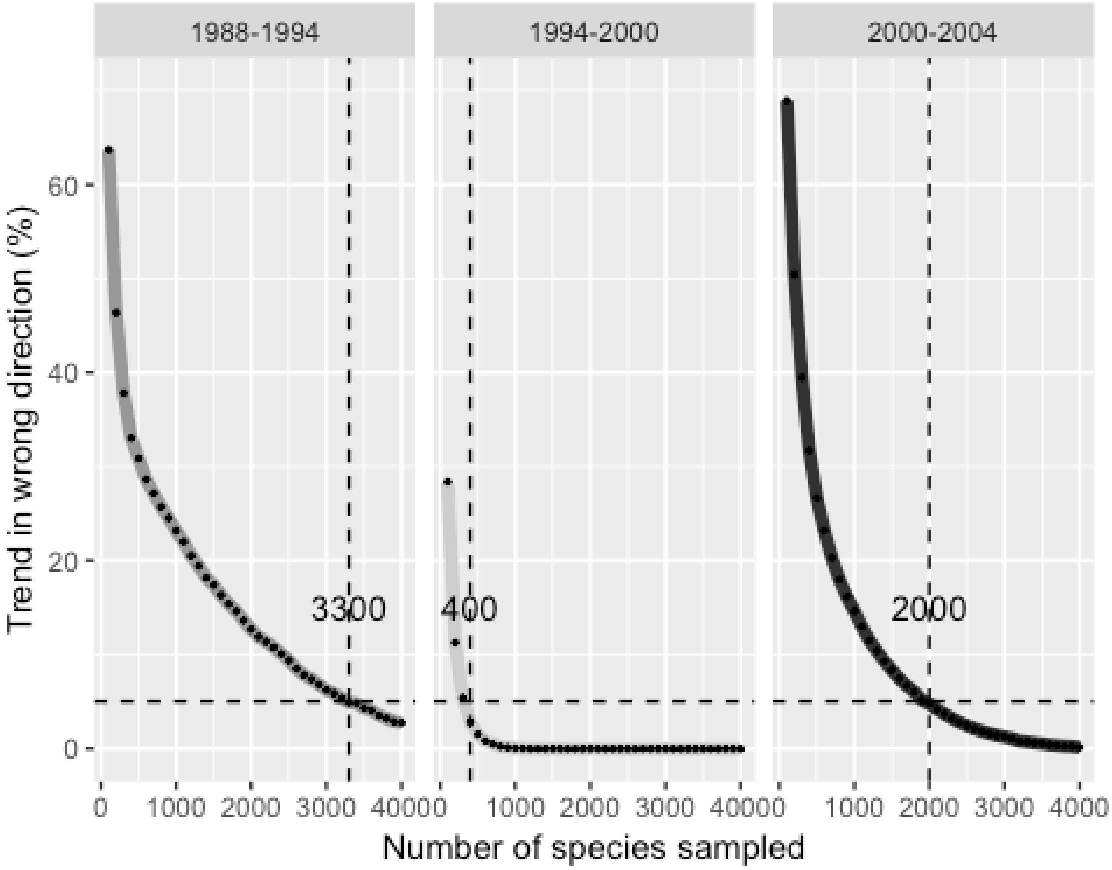
```
results = list()
i = 1
# Extract metrics from results..
for (samp in samplist) {
 # Get data
 res_filename = paste("50000sRLI_Birlife", samp, "sp.csv", sep="")
 data <- read.csv(res_filename, row.names = 1)

 all_assessments = data[, 1:4]

 resB1 <- apply(all_assessments, 1, get_slope, idx=c(1, 2))
 resB2 <- apply(all_assessments, 1, get_slope, idx=c(2, 3))
 resB3 <- apply(all_assessments, 1, get_slope, idx=c(3, 4))

 res_data = list()
 trend_list = list(resB1, resB2, resB3)
 trend_names = list("(88, 94)", "(94, 00)", "(00, 04)")
 n_assessments = list("Two","Two","Two")

 for (j in 1:3) {
 trends = trend_list[[j]]

 res_data[[j]] = data.frame(
 trend = mean(trends),
 trend_upr = quantile(trends, probs = 0.975),
```

```
 trend_lwr = quantile(trends, probs = 0.025),
 perc_wrong = sum(trends >= 0)/length(trends)*100,
 label = trend_names[[j]],
 n_ass = n_assessments[[j]],
 samp_size = samp,
 ass_years = paste(trend_names[j])
 )
 }
 res_data = do.call(rbind, res_data)

 results[[i]] = res_data
 i = i + 1
}
results_df_c = do.call(rbind, results)
write.csv(results_df_c, "BirdLife_RLI_updated_SRLI.csv")
```

under this strickter metric of correct trend detection, we can visualize how it impacts sample size of the same data set

```
results_df_c <-read.csv("BirdLife_RLI_updated_SRLI.csv")
results_df_c$n_ass_o = factor(results_df_c$ass_years, levels = c("(88,
94)", "(94, 00)", "(00, 04)"), labels = c("1988-1994", "1994-2000",
"2000-2004"))
test_c = ddply(results_df_c, .(n_ass_o), summarize, threshold =
samp_size[min(which(perc_wrong <= 5))])


# Plot line-graphs of % of incorrect slopes...
perc_plot <- ggplot(results_df_c, aes(x=samp_size, y=perc_wrong,
color=ass_years)) +
 geom_line(size=2) +
 geom_point(size=0.5, color="black") +
 geom_hline(yintercept=5, linetype="dashed") +
 ylab("Trend in wrong direction (%)") +
 xlab("Number of species sampled") +
 ylim(0,70) +
 theme(legend.position='none') +
```

```
 scale_colour_grey("Replication") +
 facet_grid(~n_ass_o, scales='free') +
 geom_vline(data = test_c, aes(xintercept=threshold), color="black",
linetype="dashed") +
 geom_text(data = test_c, aes(x=threshold, label=threshold, y=15),
color="black")


perc_plot
```



If we were just replicating the SRLI with the data available at its inception (2007) and updating it to meet current standards, the recommended sample size would be 3300 sp. However, when applying this updated approach to the data currently available we get:

2) Updating and broadening the SRLI

replicating the SRLI with the data available at its inception (2007) and updating it to meet current standards, reveiled that the recommended sample size would be

3300 sp. However, when applying this updated approach to the data currently available we get:

Birds SRLI

With red list data available for Birds in 2019

Analysing all comprehensive assessments currently available

```
#from the dataset used by BirdLife in Jan 2017
updated_bird_data = read.csv("Bird_RLI.csv")

##we remove all data deficient species
updated_bird_data = subset (updated_bird_data, X1988 != "DD")
updated_bird_data = subset (updated_bird_data, X1994 != "DD")
updated_bird_data = subset (updated_bird_data, X2000 != "DD")
updated_bird_data = subset (updated_bird_data, X2004 != "DD")
updated_bird_data = subset (updated_bird_data, X2008 != "DD")
updated_bird_data = subset (updated_bird_data, X2012 != "DD")
updated_bird_data = subset (updated_bird_data, X2016 != "DD")

##we calculated the updated RLI values for all available years
n_expe = sum(updated_bird_data$X1988 == "EX" | updated_bird_data$X1988
== "CR (PE)" | updated_bird_data$X1988 == "CR(PE)")
number_nonex = sum(updated_bird_data$X1988 != "DD") - n_expe

rli1988 = updated_RLI(updated_bird_data$X1988, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
rli1994 = updated_RLI(updated_bird_data$X1994, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
rli2000 = updated_RLI(updated_bird_data$X2000, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
rli2004 = updated_RLI(updated_bird_data$X2004, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
rli2008 = updated_RLI(updated_bird_data$X2008, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
rli2012 = updated_RLI(updated_bird_data$X2012, N_EX_YR1 = n_expe,
```

```
N_EXTANT_YR1 = number_nonex)
rli2016 = updated_RLI(updated_bird_data$X2016, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)

Birdupdated_RLI <- data.frame(rli1988=rli1988,
 rli1994=rli1994,
 rli2000=rli2000,
 rli2004=rli2004,
 rli2008=rli2008,
 rli2012=rli2012,
 rli2016=rli2016)

#which validates this formula, as it calculates the official BirdLife
RLI values
```

Calculate the slope between those simulated samples and compare them to the known slopes

```
#Setting simulation number
sim = 50000
#Increasing sample size
samplist = seq(100, 11000, by=100)
units<-1:length(updated_bird_data$binomial)
samp <- nrow(updated_bird_data)

# loop to calculate RLI values
for (samp in samplist) {
 results = list()
 # random samples
 for (i in 1:sim) {
 random.sample<-sample(units,samp)
 random.birds<-updated_bird_data[random.sample,]

 n_expe = sum(random.birds$X1988 == "EX" | random.birds$X1988 == "CR
(PE)" | random.birds$X1988 == "CR(PE)")
number_nonex = sum(random.birds$X1988 != "DD") - n_expe
```

```
 #using the currently used RLI formula
 SRLI1988 <- updated_RLI(random.birds$X1988, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
 SRLI1994 <- updated_RLI(random.birds$X1994, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
 SRLI2000 <- updated_RLI(random.birds$X2000, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
 SRLI2004 <- updated_RLI(random.birds$X2004, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
 SRLI2008 <- updated_RLI(random.birds$X2008, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
 SRLI2012 <- updated_RLI(random.birds$X2012, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)
 SRLI2016 <- updated_RLI(random.birds$X2016, N_EX_YR1 = n_expe,
N_EXTANT_YR1 = number_nonex)

 # store results for each year of assessment for this sample
 this_result = data.frame(SRLI1988=SRLI1988,
 SRLI1994=SRLI1994,
 SRLI2000=SRLI2000,
 SRLI2004=SRLI2004,
 SRLI2008=SRLI2008,
 SRLI2012=SRLI2012,
 SRLI2016=SRLI2016)

 results[[i]] = this_result
 }

 results_df = rbindlist(results)
 # writes out to a file
 write.csv(results_df, file=paste("bird_sRLIs", samp, "sp.csv", sep=""))
}
```

We can use the updated SRLI approach

```
results = list()
i = 1
```

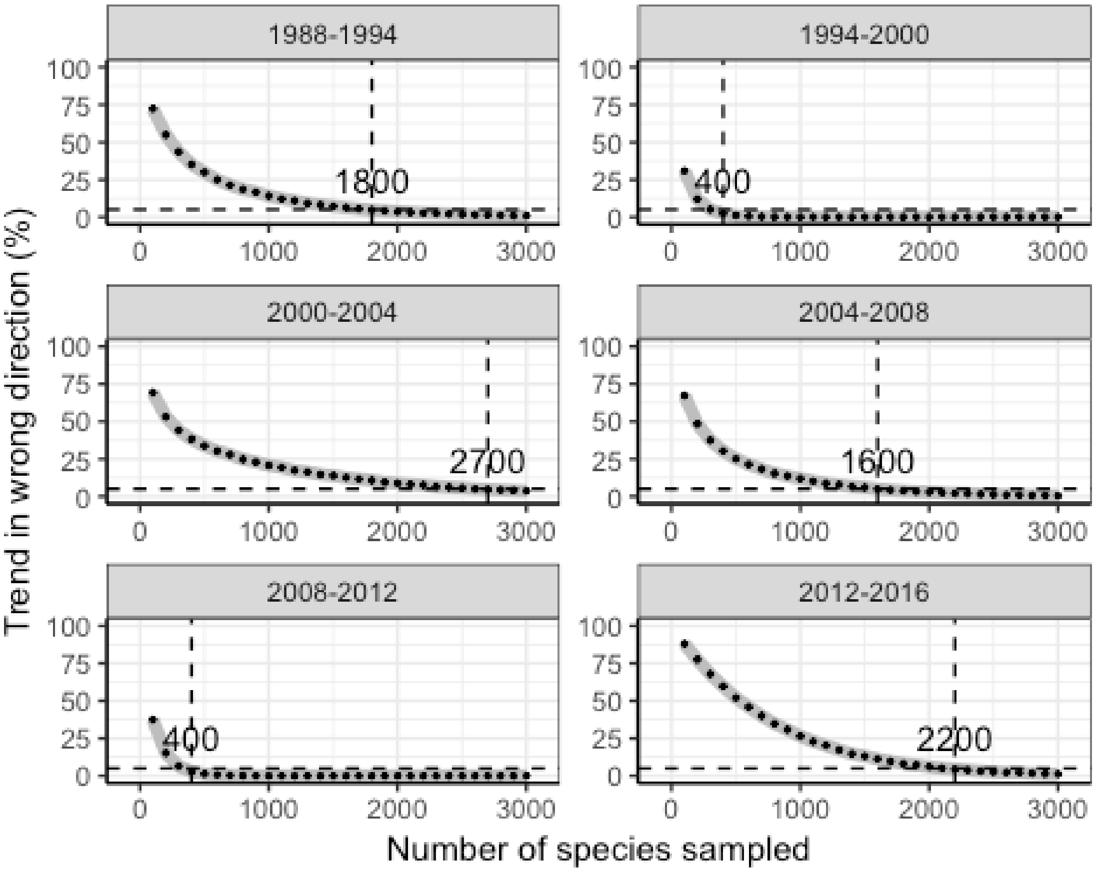
```
samplist = seq(100, 3000, by=100)
units<-1:length(updated_bird_data$binomial)
samp <- nrow(updated_bird_data)

# for each samp size
for (samp in samplist) {
 # Get data from the csv's with the SRLI simuations created earlier
 data <- read.csv(paste("bird_sRLIs", samp, "sp.csv", sep=""))
 #only the collumns with assessement year data
 all_assessments = data[, 2:8]

 #calculate the slopes by apply-ing the function get_slope to
all_assessments (with a margin 1)
 #once all_assessments is now made up of only assessment data[, 2:8];
idx=c(1, 2) is actually data for 88 and 94
 slope88_94 <- apply(all_assessments, 1, get_slope, idx=c(1, 2))
 slope94_00 <- apply(all_assessments, 1, get_slope, idx=c(2, 3))
 slope00_04 <- apply(all_assessments, 1, get_slope, idx=c(3, 4))
 slope04_08 <- apply(all_assessments, 1, get_slope, idx=c(4, 5))
 slope08_12 <- apply(all_assessments, 1, get_slope, idx=c(5, 6))
 slope12_16 <- apply(all_assessments, 1, get_slope, idx=c(6, 7))

 #name them
 trend_list = list(slope88_94, slope94_00, slope00_04, slope04_08,
slope08_12, slope12_16)
 trend_names = list("(88, 94)", "(94, 00)", "(00, 04)", "(04, 08)",
"(08, 12)", "(12, 16)")
 n_assessments = list("1988-1994","1994-2000","2000-2004","2004-
2008","2008-2012", "2012-2016")

 res_data = list()

# Place the metrics of this together
 for (j in 1:length(trend_list)) {
 trends = trend_list[[j]]
```

```
 res_data[[j]] = data.frame(
 trend = mean(trends),
 perc_wrong = sum(trends >= 0)/length(trends)*100,
 n_ass = n_assessments[[j]],
 samp_size = samp)
 }

 res_data = rbindlist(res_data)
 results[[i]] = res_data
 i = i + 1
}

results_birds = rbindlist(results)
write.csv(results_birds, "birds_updated_SRLI.csv")
```

Visualise the updated and revised SRLI results for birds

```
results_b <- read.csv("birds_updated_SRLI.csv")
results_b$n_ass_o = factor(results_b$ass_years, levels = c("(88, 94)",
"(94, 00)", "(00, 04)", "(04, 08)", "(08, 12)", "(12, 16)"), labels =
c("1988-1994","1994-2000","2000-2004","2004-2008","2008-2012", "2012-
2016"))
test_d = ddply(results_b, .(n_ass), summarize, threshold =
samp_size[min(which(perc_wrong <= 5))])

perc_plot_b <- ggplot(results_b, aes(x=samp_size, y=perc_wrong)) +
 geom_line(size=2, color="grey") +
 geom_point(size=0.5, color="black") +
 geom_hline(yintercept=5, linetype="dashed") +
 geom_vline(data = test_d, aes(xintercept=threshold), color="black",
linetype="dashed") +
 geom_text(data = test_d, aes(x=threshold, label=threshold, y=25),
color="black") +
 ylab("Trend in wrong direction (%)") +
 xlab("Number of species sampled") +
 xlim(-100, 3100) +
 ylim(0,100) +
```

```
scale_colour_discrete("Assessments") +
facet_wrap(~n_ass, ncol=2, scales='free') +
theme_bw() +
theme(axis.line=element_line(), legend.position="none")


perc_plot_b
```



Amphibians SRLI

Using available red list data used for the Amphibian RLI

Analysing the two comprehensive assessments currently available

```
amph<-read.csv("Amphibian_RLI.csv")


# Drop all DD species
amph <- subset(amph, RL_cat_80 != "DD")
amph <- subset(amph, RL_cat_04 != "DD")
```

We can use the updated SRLI approach

```
# If we set the sam to be the number of amphibians (all rows)
units<-1:length(amph$Species)
#set sample size
sim = 50000
samp <- nrow(amph)
samplist = c(seq(10, 90, by=10), seq(100, 500, by=100))

for (samp in samplist) {
 # Loop to calculate RLI values

 results_a = list()
 for (i in 1:sim) {
 random.sample<-sample(units,samp)
 random.amph<-amph[random.sample,]

 amph_n_expe = sum(random.amph$RL_cat_80 == "EX" | random.amph$RL_cat_80
== "CR (PE)" | random.amph$RL_cat_80 == "CR(PE)")
 amph_number_nonex = sum(random.amph$RL_cat_80 != "DD") - amph_n_expe

 RLI1980 <- updated_RLI(random.amph$RL_cat_80, N_EX_YR1 = amph_n_expe,
N_EXTANT_YR1 = amph_number_nonex)
 RLI2004 <- updated_RLI(random.amph$RL_cat_04, N_EX_YR1 = amph_n_expe,
N_EXTANT_YR1 = amph_number_nonex)



 # store results for each year of assessment for this sample
 this_result = data.frame(RLI1980=RLI1980, RLI2004=RLI2004)
 results_a[[i]] = this_result
 }
 results_a_df = rbindlist(results_a)

 # writes out to a file
 write.csv(results_a_df, file=paste("amph_sRLIs", samp, "sp.csv",
```

```
sep=""))
}
```

Calculate the slope between those simulated samples and compare them to the known slope

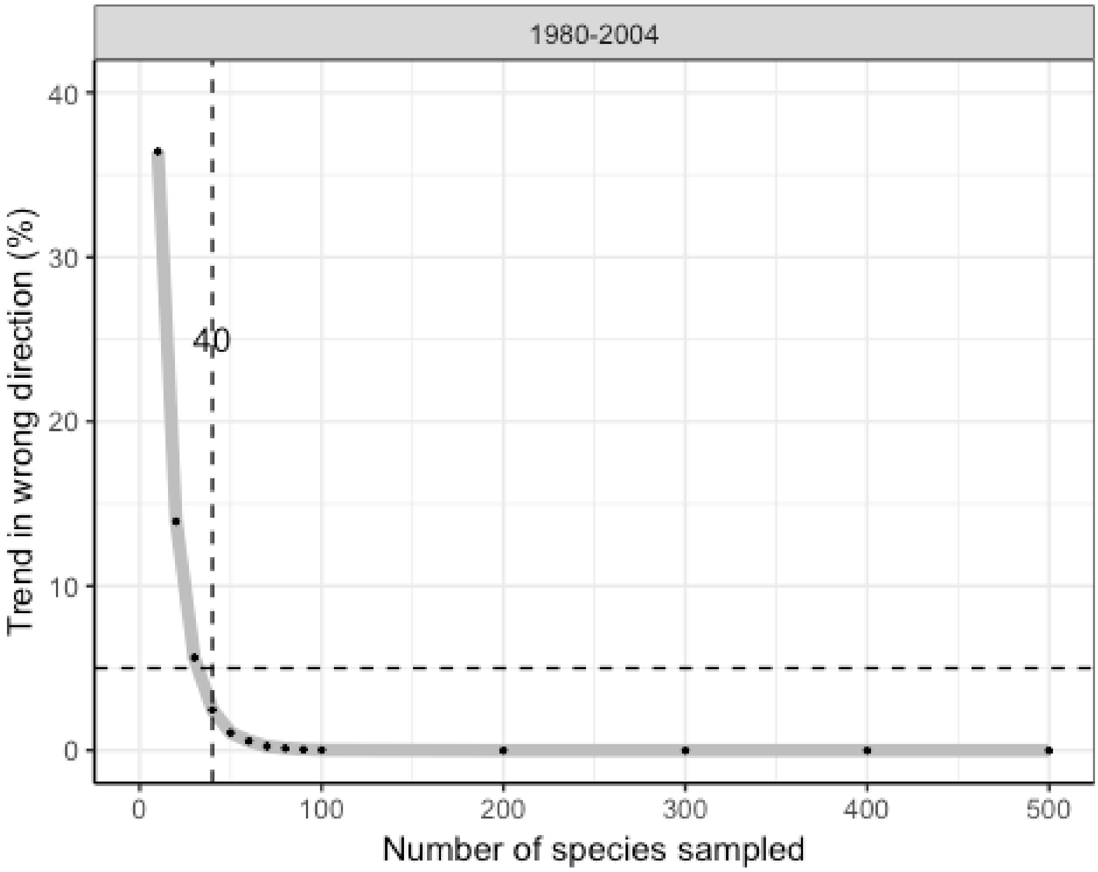
```
results = list()
i = 1
sim = 50000
samp <- nrow(amph)
samplist = c(seq(10, 90, by=10), seq(100, 500, by=100))

# similarly to what was done for birds
#for each samp size
for (samp in samplist) {
 # Get data
 data <- read.csv(paste("amph_sRLIs", samp, "sp.csv", sep=""))
 #only the collumns with assessement year data
 all_assessments = data[, 2:3]

 #calculate the slopes by applying the function get_slope to
all_assessments (with a margin 1)
 slope80_04 <- apply(all_assessments, 1, get_slope, idx=c(1, 2))

#name them
 trend_list = list(slope80_04)
 trend_names = list("(80, 04)")
 n_assessments = list("1980-2004")

 res_data = list()

#place the metrics together
 for (j in 1:length(trend_list)) {
 trends = trend_list[[j]]
 res_data[[j]] = data.frame(
 trend = mean(trends),
 trend_upr = quantile(trends, probs = 0.975),
```

```
 trend_lwr = quantile(trends, probs = 0.025),
 perc_wrong = sum(trends >= 0)/length(trends)*100,
 label = trend_names[[j]],
 n_ass = n_assessments[[j]],
 samp_size = samp,
 ass_years = paste(trend_names[j])
 )
 }

 res_data = rbindlist(res_data)
 results[[i]] = res_data
 i = i + 1
}

results_amph = rbindlist(results)
write.csv(results_amph, "amphibians_updated_SRLI.csv")
```

Visualise the updated and revised SRLI results for amphibians

```
results_amph <- read.csv("amphibians_updated_SRLI.csv")

test_d = ddply(results_amph, .(n_ass), summarize, threshold =
samp_size[min(which(perc_wrong <= 5))])

perc_plot2 <- ggplot(results_amph, aes(x=samp_size, y=perc_wrong)) +
 geom_line(size=2, color="grey") +
 geom_point(size=0.5, color="black") +
 geom_hline(yintercept=5, linetype="dashed") +
 geom_vline(data = test_d, aes(xintercept=threshold), color="black",
linetype="dashed") +
 geom_text(data = test_d, aes(x=threshold, label=threshold, y=25),
color="black") +
 ylab("Trend in wrong direction (%)") +
 xlab("Number of species sampled") +
 xlim(0, 500) +
 ylim(0,40) +
 scale_colour_discrete("Assessments") +
```

```
  facet_wrap(~n_ass, ncol=2, scales='free') +
  theme_bw() +
  theme(axis.line=element_line(), legend.position="none")


perc_plot2
```



Mammals SRLI

Using available red list data used for the Mammal RLI

Analysing the two comprehensive assessments currently available

```
#from the dataset available for mammals
mammal_data <- read.csv("Mammal_RLI.csv")


mammal_data = mammal_data[, 7:8]
##we remove all data deficient species
```

```
mammal_data = subset (mammal_data, X1996.RL.cat != "DD")
mammal_data = subset (mammal_data, X2008.RL.cat != "DD")
```

We can use the updated SRLI approach

```
units<-1:length(mammal_data$X2008.RL.cat)
sim = 50000

#set sample size
samp <- nrow(mammal_data)
samplist = seq(100, 500, by=100)

for (samp in samplist) {
 # loop to calculate RLI values

 results_m = list()
 for (i in 1:sim) {
 random.sample<-sample(units,samp)
 random.mammal<-mammal_data[random.sample,]

 mammal_n_expe = sum(random.mammal$X1996.RL.cat == "EX" |
random.mammal$X1996.RL.cat == "CR (PE)" | random.mammal$X1996.RL.cat ==
"CR(PE)")
 mammal_number_nonex = sum(random.mammal$X1996.RL.cat != "DD") -
mammal_n_expe

 RLI1996 <- updated_RLI(random.mammal$X1996.RL.cat, N_EX_YR1 =
mammal_n_expe, N_EXTANT_YR1 = mammal_number_nonex)
 RLI2008 <- updated_RLI(random.mammal$X2008.RL.cat, N_EX_YR1 =
mammal_n_expe, N_EXTANT_YR1 = mammal_number_nonex)



 # store results for each year of assessment for this sample
 this_result = data.frame(RLI1996=RLI1996, RLI2008=RLI2008)
 results_m[[i]] = this_result
 }
 results_m_df = rbindlist(results_m)
```

```
 # writes out to a file
 write.csv(results_m_df, file=paste("mammal_sRLIs", samp, "sp.csv",
sep=""))

}
```

Calculate the slope between those simulated samples and compare them to the known slope

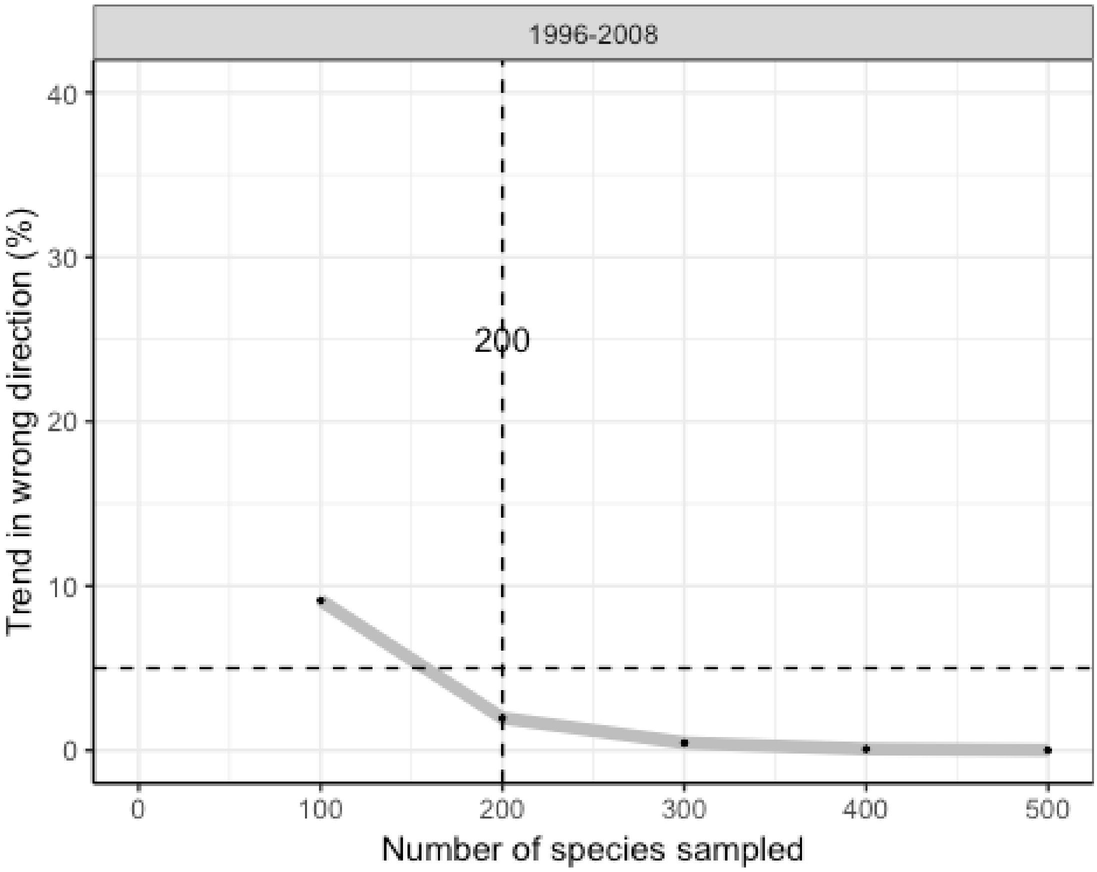
```
results = list()
i = 1
sim = 50000
samp <- nrow(mammal_data)
samplist = seq(100, 500, by=100)
#for each samp size
for (samp in samplist) {
 # Get data
 data <- read.csv(paste("mammal_sRLIs", samp, "sp.csv", sep=""))
 #only the collumns with assessement year data
 all_assessments = data[, 2:3]

 #calculate the slopes by apply-ing the function get_slope to
all_assessments (with a margin 1)
 slope96_08 <- apply(all_assessments, 1, get_slope, idx=c(1, 2))

#name them
 trend_list = list(slope96_08)
 trend_names = list("(96, 08)")
 n_assessments = list("1996-2008")

 res_data = list()

#place the metrics together
 for (j in 1:length(trend_list)) {
 trends = trend_list[[j]]
 res_data[[j]] = data.frame(
```

```
 trend = mean(trends),
 trend_upr = quantile(trends, probs = 0.975),
 trend_lwr = quantile(trends, probs = 0.025),
 perc_wrong = sum(trends >= 0)/length(trends)*100,
 label = trend_names[[j]],
 n_ass = n_assessments[[j]],
 samp_size = samp,
 ass_years = paste(trend_names[j])
 )
 }

 res_data = rbindlist(res_data)
 results[[i]] = res_data
 i = i + 1
}

results_mammal = rbindlist(results)
write.csv(results_mammal, "mammal_updated_SRLI.csv")
```

Visualise the SRLI results for mammals for the first time

```
results_mammal <- read.csv("mammal_updated_SRLI.csv")
test_d = ddply(results_mammal, .(n_ass), summarize, threshold =
samp_size[min(which(perc_wrong <= 5))])

perc_plot2 <- ggplot(results_mammal, aes(x=samp_size, y=perc_wrong)) +
 geom_line(size=2, color="grey") +
 geom_point(size=0.5, color="black") +
 geom_hline(yintercept=5, linetype="dashed") +
 geom_vline(data = test_d, aes(xintercept=threshold), color="black",
linetype="dashed") +
 geom_text(data = test_d, aes(x=threshold, label=threshold, y=25),
color="black") +
 ylab("Trend in wrong direction (%)") +
 xlab("Number of species sampled") +
 xlim(0, 500) +
 ylim(0,40) +
```

```
  scale_colour_discrete("Assessments") +
  facet_wrap(~n_ass, ncol=2, scales='free') +
  theme_bw() +
  theme(axis.line=element_line(), legend.position="none")


perc_plot2
```



Corals SRLI

Using available red list data used for the Coral RLI

Analysing the two comprehensive assessments currently available

```
#from the dataset available for corals
coral_data <- read.csv("Coral_RLI.csv")

#just get the data we need
coral_data$backcast_perc_red.<- NULL
```

```
coral_data = coral_data[, 5:6]

#Name the collums with the years they represent
colnames(coral_data)[colnames(coral_data)=="Past_Cat"] <- "x1998"
colnames(coral_data)[colnames(coral_data)=="Current_Cat"] <- "x2008"
coral_data<- coral_data[,c("x1998", "x2008")]

##we remove all data deficient species
coral_data = subset (coral_data, x1998 != "DD")
coral_data = subset (coral_data, x2008 != "DD")
```

We can use the updated SRLI approach

```
# If we set the sam to be the number of amphibians (all rows)
units<-1:length(coral_data$x1998)
#set sample size
sim = 50000
samp <- nrow(coral_data)
samplist = c(seq(1, 9, by=1), seq(10, 100, by=10))

for (samp in samplist) {
 # loop to calculate RLI values

 results_c = list()
 for (i in 1:sim) {
 random.sample<-sample(units,samp)
 random.coral<-coral_data[random.sample,]

 coral_n_expe = sum(coral_data$x1998 == "EX" | coral_data$x1998 == "CR
(PE)" | coral_data$x1998 == "CR(PE)")
 coral_number_nonex = sum(coral_data$x1998 != "DD") - coral_n_expe

 RLI1998 <- updated_RLI(random.coral$x1998, N_EX_YR1 = coral_n_expe,
N_EXTANT_YR1 = coral_number_nonex)
 RLI2008 <- updated_RLI(random.coral$x2008, N_EX_YR1 = coral_n_expe,
N_EXTANT_YR1 = coral_number_nonex)
```

```
 # store results for each year of assessment for this sample
 this_result = data.frame(RLI1998=RLI1998, RLI2008=RLI2008)
 results_c[[i]] = this_result
 }
 results_c_df = rbindlist(results_c)

 # writes out to a file
 write.csv(results_c_df, file=paste("coral_sRLIs", samp, "sp.csv",
sep=""))
}
```

Calculate the slope between those simulated samples and compare them to the known slope

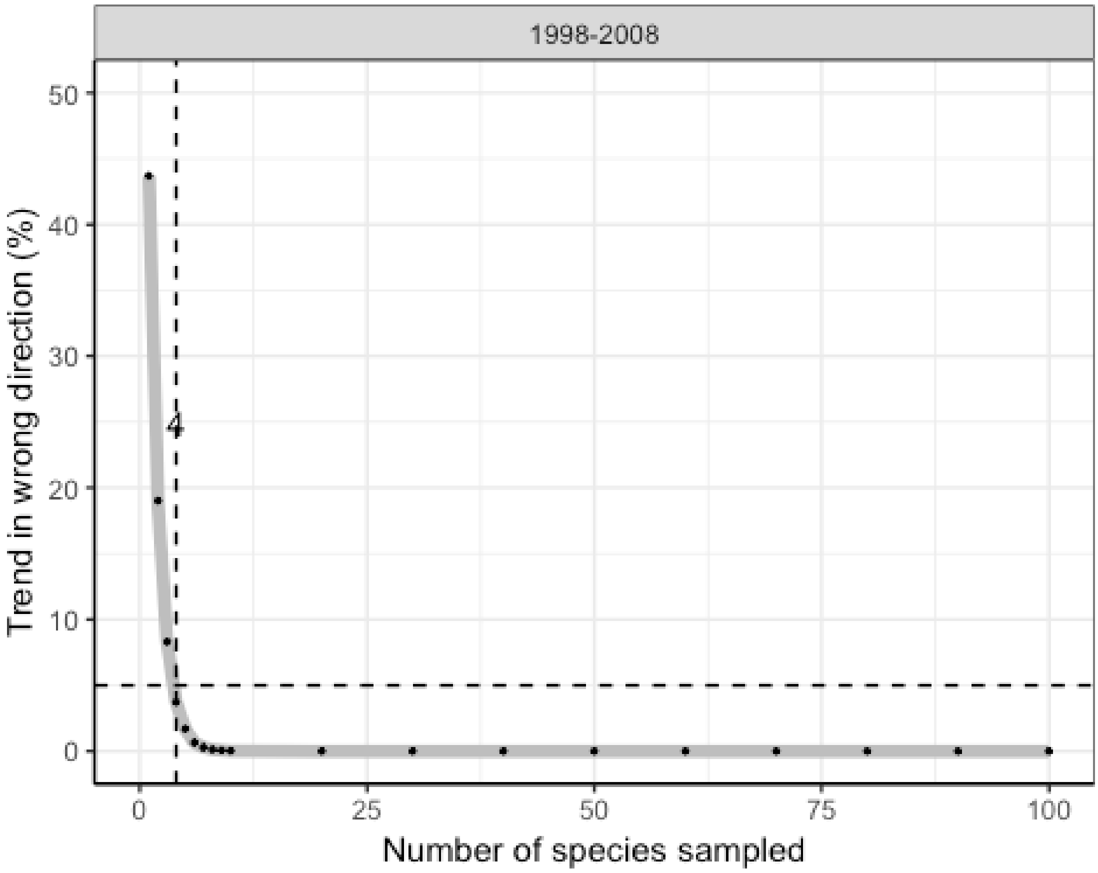
```
results = list()
i = 1
sim = 50000
samp <- nrow(coral_data)
samplist = c(seq(1, 9, by=1), seq(10, 100, by=10))

#for each samp size
for (samp in samplist) {
 # Get data
 data <- read.csv(paste("coral_sRLIs", samp, "sp.csv", sep=""))
 #only the collumns with assessement year data
 all_assessments = data[, 2:3]

 #calculate the slopes by apply-ing the function get_slope to
all_assessments (with a margin 1)

 slope98_08 <- apply(all_assessments, 1, get_slope, idx=c(1, 2))

#name them
 trend_list = list(slope98_08)
 trend_names = list("(98, 08)")
 n_assessments = list("1998-2008")
```

```
 res_data = list()

#place the metrics together
 for (j in 1:length(trend_list)) {
 trends = trend_list[[j]]
 res_data[[j]] = data.frame(
 trend = mean(trends),
 trend_upr = quantile(trends, probs = 0.975),
 trend_lwr = quantile(trends, probs = 0.025),
 perc_wrong = sum(trends >= 0)/length(trends)*100,
 label = trend_names[[j]],
 n_ass = n_assessments[[j]],
 samp_size = samp,
 ass_years = paste(trend_names[j])
 )
 }

 res_data = rbindlist(res_data)
 results[[i]] = res_data
 i = i + 1
}

results_coral = rbindlist(results)
write.csv(results_coral, "coral_updated_SRLI.csv")
```

Visualise the SRLI results for corals for the first time

```
results_coral <- read.csv("coral_updated_SRLI.csv")
test_d = ddply(results_coral, .(n_ass), summarize, threshold =
samp_size[min(which(perc_wrong <= 5))])

perc_plot3 <- ggplot(results_coral, aes(x=samp_size, y=perc_wrong)) +
 geom_line(size=2, color="grey") +
 geom_point(size=0.5, color="black") +
 geom_hline(yintercept=5, linetype="dashed") +
 geom_vline(data = test_d, aes(xintercept=threshold), color="black",
```

```
linetype="dashed") +
 geom_text(data = test_d, aes(x=threshold, label=threshold, y=25),
color="black") +
 ylab("Trend in wrong direction (%)") +
 xlab("Number of species sampled") +
 xlim(0, 100) +
 ylim(0,50) +
 scale_colour_discrete("Assessments") +
 facet_wrap(~n_ass, ncol=2, scales='free') +
 theme_bw() +
 theme(axis.line=element_line(), legend.position="none")


perc_plot3
```



Cycads SRLI

Using available red list data used for the Cycads RLI

Analysing the two comprehensive assessments currently available

```
#data wrangling
cycad_data <- read.csv("Cycad_RLI.csv")
cycad_data$`X.2`<- NULL
cycad_data$`X.3`<- NULL
cycad_data = cycad_data[, 2:4]
colnames(cycad_data) = cycad_data[1,]
cycad_data = cycad_data[-1,]

#Name the collums with the years they represent
colnames(cycad_data)[colnames(cycad_data)=="9"] <- "x2003"
colnames(cycad_data)[colnames(cycad_data)=="1"] <- "x2014"

##we remove all data deficient species
cycad_data = subset (cycad_data, x2003 != "DD")
cycad_data = subset (cycad_data, x2003 != "DD(CR)")
cycad_data = subset (cycad_data, x2003 !="DD(EN)")
cycad_data = subset (cycad_data, x2003 != "DD(LC)")
cycad_data = subset (cycad_data, x2003 != "DD(NT)")
cycad_data = subset (cycad_data, x2003 != "DD(VU)")
cycad_data = subset (cycad_data, x2003 != "(DD)VU")
```

Using the updated SRLI approach

```
units<-1:length(cycad_data$x2003)
#set sample size
sim = 50000
samp <- nrow(cycad_data)
samplist = c(seq(10, 90, by=10), seq(100, 300, by=100))

for (samp in samplist) {
 # loop to calculate RLI values

 results_cy = list()

 for (i in 1:sim) {
```

```
 random.sample<-sample(units,samp)
 random.cycad<-cycad_data[random.sample,]

 cycad_n_expe = sum(random.cycad$x2003 == "EX" | random.cycad$x2003 ==
"CR (PE)" | random.cycad$x2003 == "CR(PE)" | random.cycad$x2003 ==
"(EW)" | random.cycad$x2003 == "EW" )
 cycad_number_nonex = sum(random.cycad$x2003 != "DD") - cycad_n_expe

 RLI2003 <- updated_RLI(random.cycad$x2003, N_EX_YR1 = cycad_n_expe,
N_EXTANT_YR1 = cycad_number_nonex)
 RLI2014 <- updated_RLI(random.cycad$x2014, N_EX_YR1 = cycad_n_expe,
N_EXTANT_YR1 = cycad_number_nonex)



 # store results for each year of assessment for this sample
 this_result = data.frame(RLI2003=RLI2003, RLI2014=RLI2014)
 results_cy[[i]] = this_result
 }
 results_cycads = rbindlist(results_cy)

 # writes out to the files
 write.csv(results_cycads, file=paste("cycads_sRLIs", samp, "sp.csv",
sep=""))
}
```

Calculate the slope between those simulated samples and compare them to the known slope

```
results = list()
i = 1

units<-1:length(cycad_data$x2003)
samp <- nrow(cycad_data)
samplist = c(seq(10, 90, by=10), seq(100, 300, by=100))

#for each samp size
for (samp in samplist) {
```

```
# Get data
data <- read.csv(paste("cycads_sRLIs", samp, "sp.csv", sep=""))
#only the collumns with assessement year data
all_assessments = data[, 2:3]

 #calculate the slopes by applying the function get_slope to
all_assessments (with a margin 1)
 slope03_14 <- apply(all_assessments, 1, get_slope, idx=c(1, 2))

#name them
 trend_list = list(slope03_14)
 trend_names = list("(03, 14)")
 n_assessments = list("2003-2014")

 res_data = list()

#place the metrics together
 for (j in 1:length(trend_list)) {
 trends = trend_list[[j]]
 res_data[[j]] = data.frame(
 perc_wrong = sum(trends >= 0)/length(trends)*100,
 n_ass = n_assessments[[j]],
 samp_size = samp)
 }

 res_data = rbindlist(res_data)
 results[[i]] = res_data
 i = i + 1

}

results_cycads = rbindlist(results)
write.csv(results_cycads, "cycad_updated_SRLI.csv")
```

Visualise the SRLI results for cycads for the first time

```r
results_cycads <- read.csv("cycad_updated_SRLI.csv")
test_d = ddply(results_cycads, .(n_ass), summarize, threshold =
samp_size[min(which(perc_wrong <= 5))])

perc_plot4 <- ggplot(results_cycads, aes(x=samp_size, y=perc_wrong)) +
 geom_line(size=2, color="grey") +
 geom_point(size=0.5, color="black") +
 geom_hline(yintercept=5, linetype="dashed") +
 geom_vline(data = test_d, aes(xintercept=threshold), color="black",
linetype="dashed") +
 geom_text(data = test_d, aes(x=threshold, label=threshold, y=25),
color="black") +
 ylab("Trend in wrong direction (%)") +
 xlab("Number of species sampled") +
 xlim(0, 300) +
 ylim(0, 20) +
 scale_colour_discrete("Assessments") +
 facet_wrap(~n_ass, ncol=2, scales='free') +
 theme_bw() +
 theme(axis.line=element_line(), legend.position="none")


perc_plot4
```

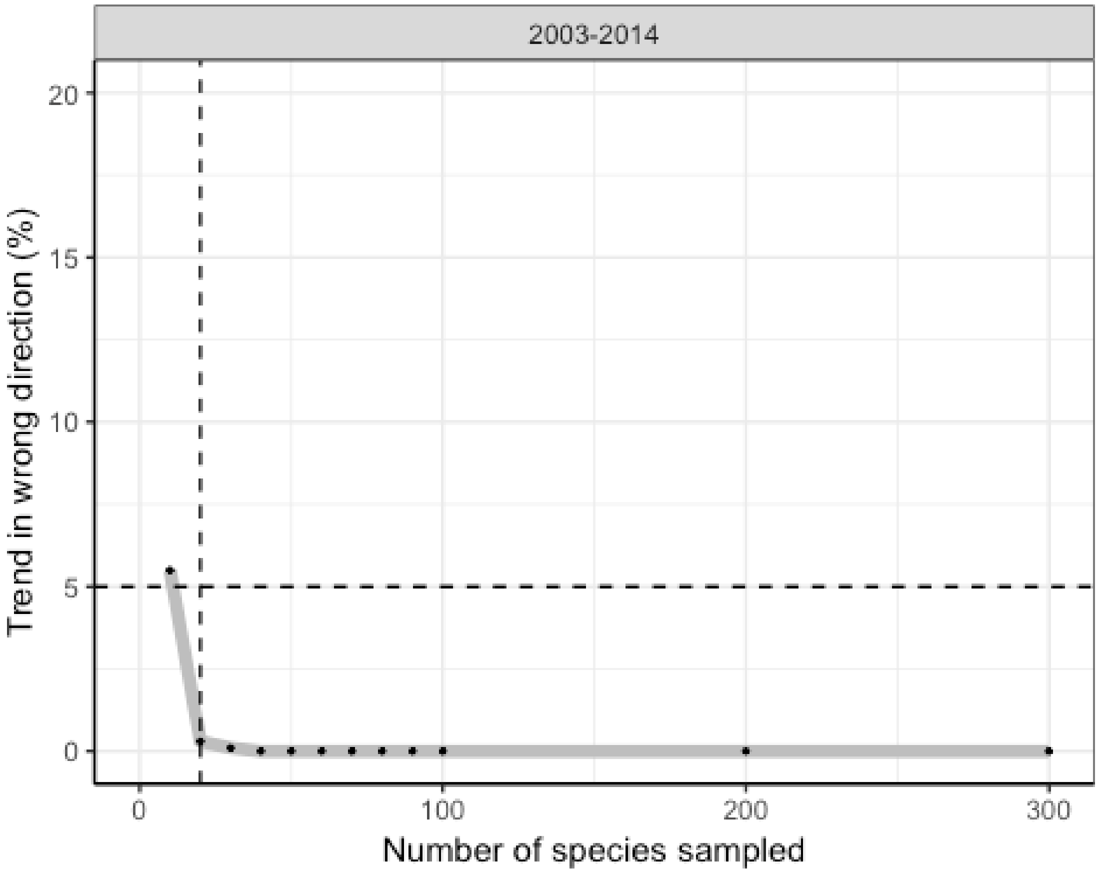


Figure 2.1 The combination of the individual plots of each taxa into a grid, produces Fig.2.1

3) Slope

Slopes

We also measured what sample was needed to correctly detect change between two slopes (rather than slope direction)

Using the bird data (as the only group with more than one reassessment - two slopes), under the current BIrdLife RLI formula and under our updated SRLI protocol.

```
# Calculate the slopes (aka difference betwween RLIs)
RLIdiff <- diff(as.numeric(Birdupdated_RLI))
# Calculate if they are flat, accelerating or decelarating (aka
difference between slopes)
SlopeDiff <- diff(RLIdiff)
```

Calculate these metrics for increasing sample size, from 100 species to a maximum of 3,000 at increments of 100.

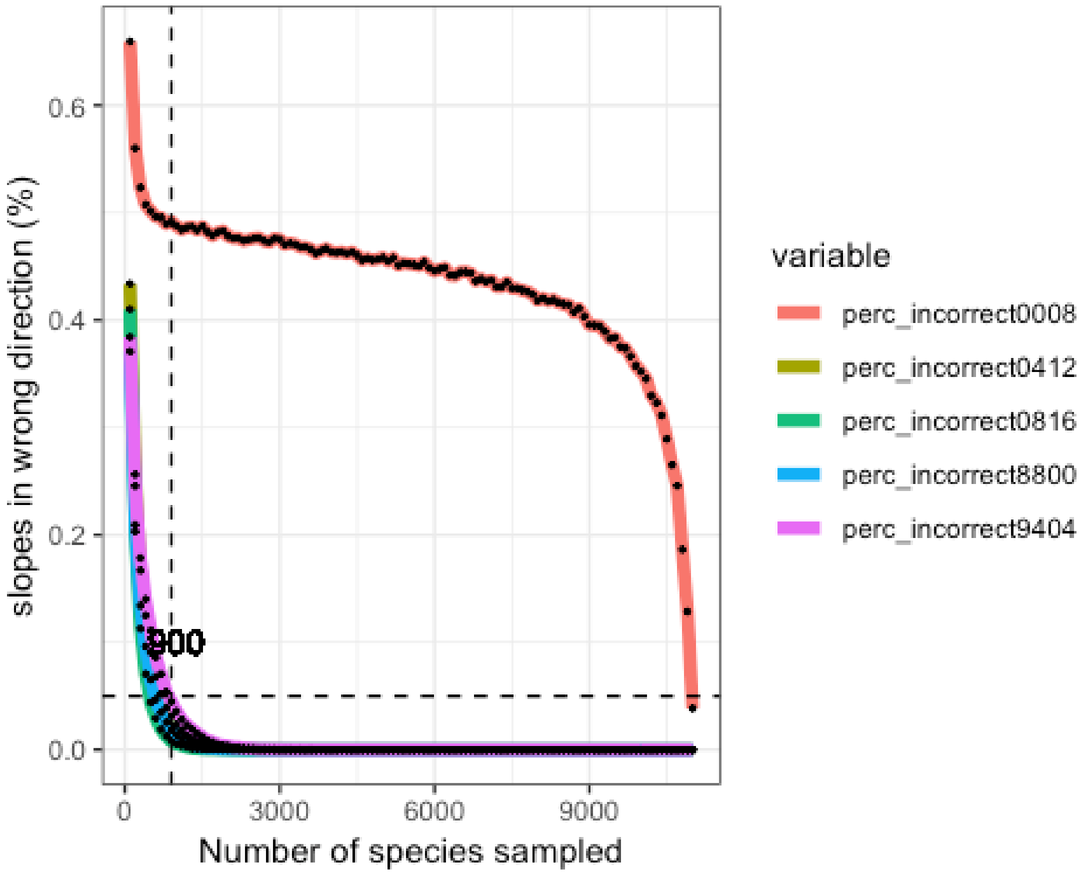
```
resultslope = list()
i=1
samplist = seq(100, 11000, by=100)



for (samp in samplist) {
 Sampledata <- read.csv(file=paste("bird_sRLIs", samp, "sp.csv",
sep=""), row.names=1)
 #How many samples calculate the proportion of samples that correctly
detect the slope
 SRLIdiff <- t(matrix(apply(Sampledata, 1, diff), nrow=6, ncol=50000))

 #Difference between sample slopes
 SampSlopeDiff <- t(apply(SRLIdiff, 1, diff))

 #calculate the number of samples that correctly detect the differences
between different slopes

 n_incorrect8800 = sum (sign(SampSlopeDiff[, 1]) != sign(SlopeDiff[1]))
 n_incorrect9404 = sum (sign(SampSlopeDiff[, 2]) != sign(SlopeDiff[2]))
 n_incorrect0008 = sum (sign(SampSlopeDiff[, 3]) != sign(SlopeDiff[3]))
 n_incorrect0412 = sum (sign(SampSlopeDiff[, 4]) != sign(SlopeDiff[4]))
 n_incorrect0816 = sum (sign(SampSlopeDiff[, 5]) != sign(SlopeDiff[5]))

#and measure what percentage of wrong detection those values represent
 perc_incorrect8800=n_incorrect8800/nrow(SampSlopeDiff)
 perc_incorrect9404=n_incorrect9404/nrow(SampSlopeDiff)
 perc_incorrect0008=n_incorrect0008/nrow(SampSlopeDiff)
 perc_incorrect0412=n_incorrect0412/nrow(SampSlopeDiff)
 perc_incorrect0816=n_incorrect0816/nrow(SampSlopeDiff)

 results[[i]] = data.frame(perc_incorrect8800, perc_incorrect9404,
perc_incorrect0008, perc_incorrect0412, perc_incorrect0816)
```

```
 i = i+1
}

resultslope <- rbindlist(results)
resultslope$samplesize = samplist
resultslope = melt(resultslope[, c(1:5, 6)], id.vars = "samplesize")
write.csv(resultslope, "sample_slope11000.csv")
```

Figure 2.2

When we plot those results

```
resultslope <- read.csv("sample_slope11000.csv")

ggplot(resultslope, aes(x=samplesize, y=value, group=variable,
colour=variable)) +
geom_line(size=2, aes(group = variable)) +
geom_point(size=0.5, color="black") +
geom_hline(yintercept=0.05, linetype="dashed") +
geom_vline(xintercept=900, linetype="dashed") +
geom_text(aes(x=1000, label=900, y=0.1), color="black") +
ylab("slopes in wrong direction (%)") +
xlab("Number of species sampled") +
theme_bw()
```



Only when increasing sample size considerably, do we find that "perc_incorrect0008" only crosses the 0.5 threshold at around 11.000 sp (producing Figure 2.2 B)

10 year slopes

A similar analysis was made, to calculate the sample size needed if comprehensive assessments occurred with at least 10 year intervals

```
# Calculate the < 10y slopes (aka difference betwween 88-00; 00-12 and 94-04; 04-16)
subRLI8812 <- Birdupdated_RLI[c(1,3,6)]
RLIdiff8812 <- diff(as.numeric(subRLI8812))

subRLI9416 <- Birdupdated_RLI[c(2,4,7)]
RLIdiff9416 <- diff(as.numeric(subRLI9416))
# Caculate the difference between those slopes
SlopeDiff8812 <- diff(RLIdiff8812)
SlopeDiff9416 <- diff(RLIdiff9416)
```

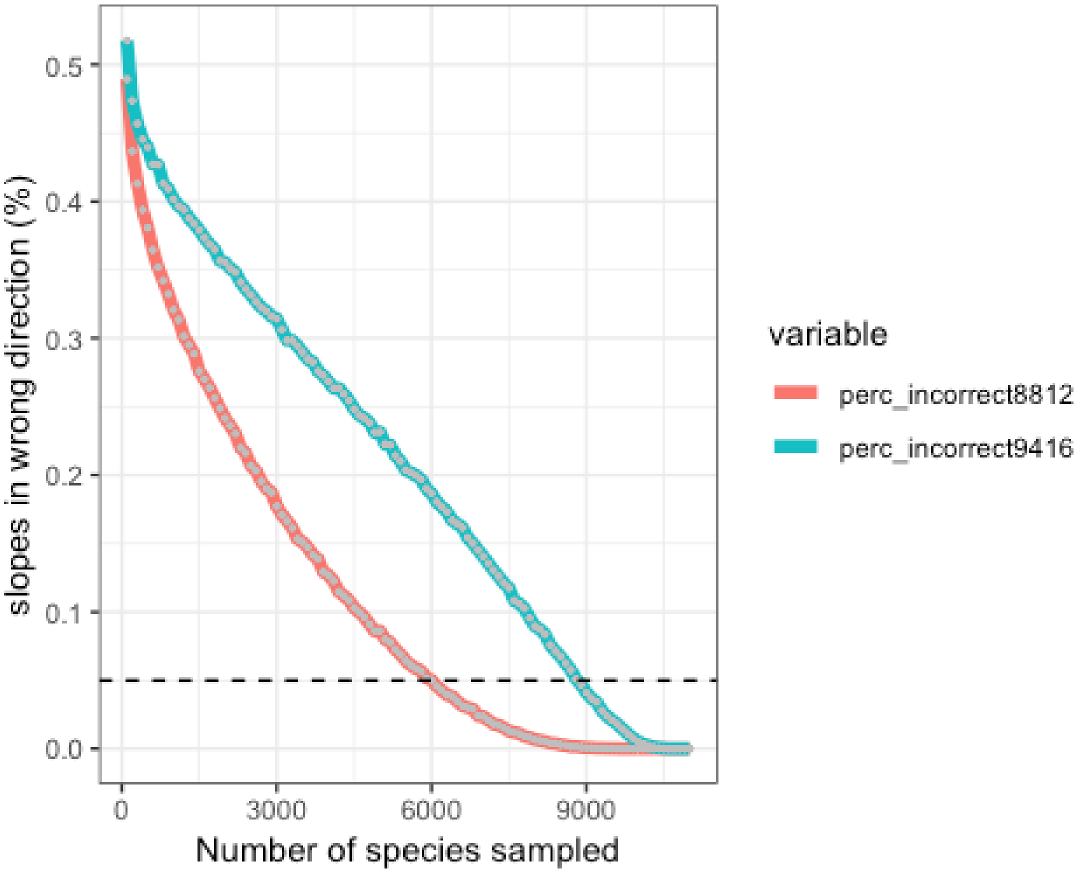
Calculate these metrics for increasing sample size, from 100 species to a maximum of 3,000 at increments of 100.

```
results10y = list()
i=1
samplist = seq(100, 11000, by=100)


for (samp in samplist) {
 Sampledata <- read.csv(file=paste("bird_sRLIs", samp, "sp.csv",
sep=""), row.names=1)
 Sampledata8812 <- Sampledata[c(1,3,6)]
 Sampledata9416 <- Sampledata[c(2,4,7)]

 #Difference between srli
 SRLIdiff8812 <- t(matrix(apply(Sampledata8812, 1, diff), nrow=2,
ncol=50000))
 SRLIdiff9416 <- t(matrix(apply(Sampledata9416, 1, diff), nrow=2,
ncol=50000))

 #Difference between sample slopes
 SampSlopeDiff8812 <- apply(SRLIdiff8812, 1, diff)
 SampSlopeDiff9416 <- apply(SRLIdiff9416, 1, diff)

 #calculate the number of samples that correctly detect the differences
between different slopes
 n_incorrect8812 = sum (sign(SampSlopeDiff8812) != sign(SlopeDiff8812))
 n_incorrect9416 = sum (sign(SampSlopeDiff9416) != sign(SlopeDiff9416))

 perc_incorrect8812=n_incorrect8812/length(SampSlopeDiff8812)
 perc_incorrect9416=n_incorrect9416/length(SampSlopeDiff9416)

 results10y[[i]] = data.frame(perc_incorrect8812, perc_incorrect9416)
 i = i+1
}
```

```
resultslope10y <- rbindlist(results10y)
resultslope10y$samplesize = samplist
resultslope10y = melt(resultslope10y[, c(1:2, 3)], id.vars =
"samplesize")
write.csv(resultslope10y, "sample_slope10y11000.csv")
```

Ploting this data shows a that a considerably large sample size of well over 3000 species would be needed

```
resultslope10y <- read.csv("sample_slope10y11000.csv")

ggplot(resultslope10y, aes(x=samplesize, y=value, group=variable,
colour=variable)) +
 geom_line(size=2, aes(group = variable)) +
 geom_point(size=0.5, color="grey") +
 geom_hline(yintercept=0.05, linetype="dashed") +
 ylab("slopes in wrong direction (%)") +
 xlab("Number of species sampled") +
 theme_bw()
```



4) Interassessment

We selectively bypassed comprehensive assessments to simulate all possible inter-assessment lengths

Using the bird data, as the only group with more than two reassessment, with the currently used RLI formula and under the updated SRLI protocol. We simulated different inter-assessment lengths as detailed in methods and summarized in Supporting information S3.

```
#using the SRLI simulation files created earlier with increasing sample size, from 100 species to a maximum of 3,000 at increments of 100.
results = list()
i = 1
sim = 50000
#Increasing sample size
samplist = seq(100, 3000, by=100)
units<-1:length(updated_bird_data$binomial)
```

```
samp <- nrow(updated_bird_data)

### Comparing all possible inter-assessement length
for (samp in samplist) {
 data <- read.csv(paste("bird_sRLIs", samp, "sp.csv", sep=""), row.names
= 1)

 all_assessments = data[, 1:7]
 #interassements of 4 years
 resA1 <- apply(all_assessments, 1, get_slope, idx=c(3, 4))
 resA2 <- apply(all_assessments, 1, get_slope, idx=c(4, 5))
 resA3 <- apply(all_assessments, 1, get_slope, idx=c(5, 6))
 resA4 <- apply(all_assessments, 1, get_slope, idx=c(6, 7))

 #interassements of 6 years
 resB1 <- apply(all_assessments, 1, get_slope, idx=c(1, 2))
 resB2 <- apply(all_assessments, 1, get_slope, idx=c(2, 3))

 #interassements of 8 years
 resC1 <- apply(all_assessments, 1, get_slope, idx=c(3, 5))
 resC2 <- apply(all_assessments, 1, get_slope, idx=c(4, 6))
 resC3 <- apply(all_assessments, 1, get_slope, idx=c(5, 7))

 #interassements of 10 years
 resD1 <- apply(all_assessments, 1, get_slope, idx=c(2, 4))

 #interassements of 12 years
 resE1 <- apply(all_assessments, 1, get_slope, idx=c(1, 3))
 resE2 <- apply(all_assessments, 1, get_slope, idx=c(3, 6))
 resE3 <- apply(all_assessments, 1, get_slope, idx=c(4, 7))

 #interassements of 14 years
 resF1 <- apply(all_assessments, 1, get_slope, idx=c(2, 5))

 #interassements of 16 years
 resG1 <- apply(all_assessments, 1, get_slope, idx=c(1, 4))
```

```
 resG2 <- apply(all_assessments, 1, get_slope, idx=c(3, 7))

 #interassements of 18 years
 resH1 <- apply(all_assessments, 1, get_slope, idx=c(2, 6))

 #interassements of 20 years
 resI1 <- apply(all_assessments, 1, get_slope, idx=c(1, 5))

 #interassements of 22 years
 resJ1 <- apply(all_assessments, 1, get_slope, idx=c(2, 7))

 #interassements of 24 years
 resK1 <- apply(all_assessments, 1, get_slope, idx=c(1, 6))

 #interassements of 28 years
 resL1 <- apply(all_assessments, 1, get_slope, idx=c(1, 7))


 #compiling the data
 trend_list = list(resA1, resA2, resA3, resA4, resB1, resB2, resC1,
resC2, resC3, resD1, resE1, resE2, resE3, resF1, resG1, resG2, resH1,
resI1, resJ1, resK1, resL1)

 #naming it according to the year on analysis
 trend_names = list( "(00, 04)", "(04, 08)", "(08, 12)", "(12, 16)",
 "(88, 94)", "(94,00)",
 "(00, 08)", "(04, 12)", "(08, 16)",
 "(94, 04)",
 "(88, 00)", "(00, 12)", "(04, 16)",
 "(94, 08)",
 "(88, 04)", "(00, 16)",
 "(94, 12)",
 "(88, 08)",
 "(94, 16)",
 "(88, 12)",
 "(88, 16)")
```

```
 #labeled by the interassessment length
 n_assessments = list("Four", "Four", "Four", "Four",
 "Six", "Six",
 "Eight", "Eight", "Eight",
 "Ten",
 "Twelve", "Twelve", "Twelve",
 "Fourteen",
 "Sixteen", "Sixteen",
 "Eighteen", "Twenty", "Twenty two", "Twenty four", "Twenty eight")

 res_data = list()

 for (j in 1:length(trend_list)){
 trends = trend_list[[j]]
 res_data[[j]] = data.frame(
 perc_wrong = sum(trends >= 0)/length(trends)*100,
 label = trend_names[[j]],
 n_ass = n_assessments[[j]],
 samp_size = samp,
 ass_years = paste(colnames(data)[j-1], "-", colnames(data)[j])
 )
 }
 res_data = rbindlist(res_data)
 results[[i]] = res_data
 i = i + 1
}
results = rbindlist(results)

write.csv(results, "inter_assessments.csv")
```

Figure S2.1

Plotting this data in individual graphs per interassessment length, produces Figure S2.1 (in Supporting information)

```
inter_results = read.csv("inter_assessments.csv")

inter_results$n_ass <- factor(inter_results$n_ass, levels = c("Four",
"Six", "Eight", "Ten", "Twelve", "Fourteen", "Sixteen", "Eighteen",
"Twenty", "Twenty two", "Twenty four", "Twenty eight" ))



test_d = ddply(inter_results, .(n_ass, label), summarize, threshold =
samp_size[min(which(perc_wrong <= 5))])

# RF: ggplot(results, aes(x=samp_size, y=perc_wrong, color=label)) +
ggplot(inter_results, aes(x=samp_size, y=perc_wrong, color=label)) +
 geom_line(size=1) +
 geom_point(size=0.5, color="black") +
 geom_hline(yintercept=5, linetype="dashed") +
 geom_dl(aes(label = label), method = list(dl.trans(x=x-0.1),
"first.points", cex = 0.6), color="black") +
 ylab("Trend in wrong direction (%)") +
 xlab("Number of species sampled") +
 xlim(-300, 3000) +
 ylim(0,100) +
 scale_colour_grey("Assessments") +
 theme_bw() +
 theme(axis.line=element_line(), legend.position="none")+
 geom_vline(data = test_d, aes(xintercept=threshold), color="black",
linetype="dashed")+
 geom_text(data = test_d, aes(x=threshold, label=threshold, y=25),
color="black") +
 facet_wrap(~n_ass, scales='free')
```

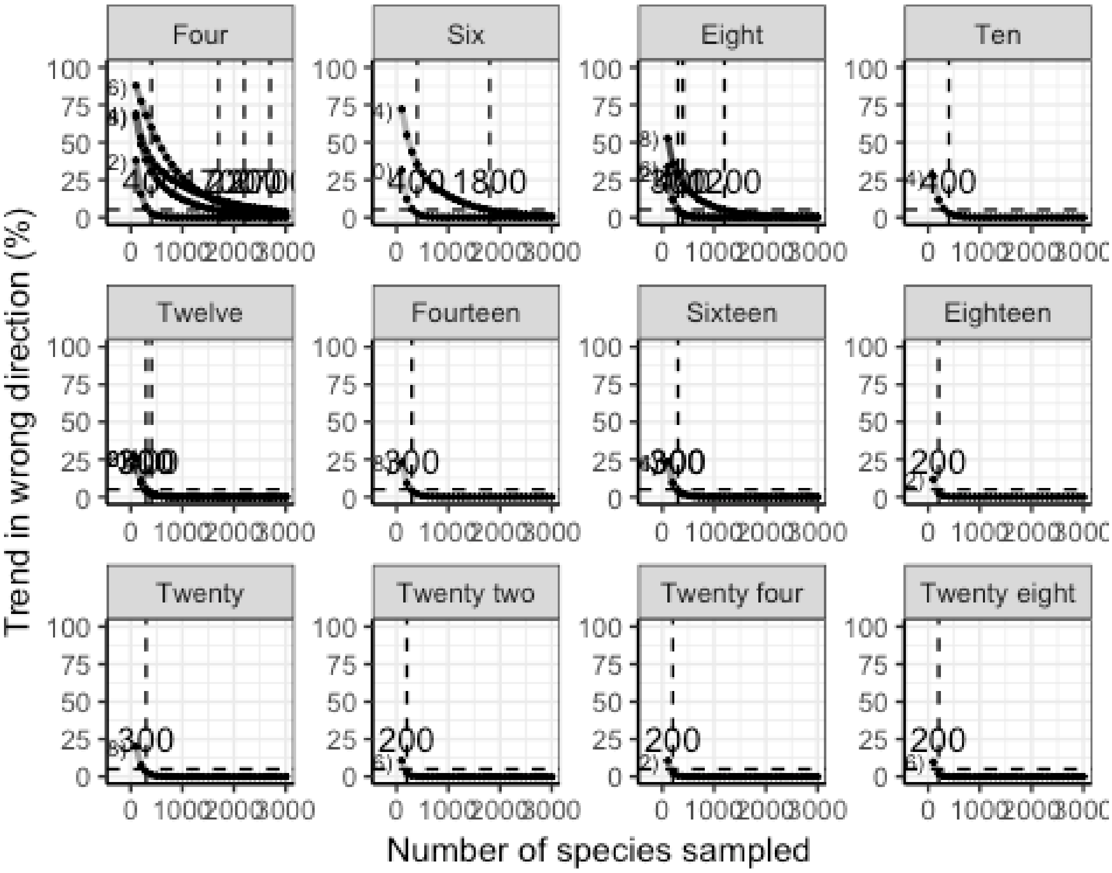
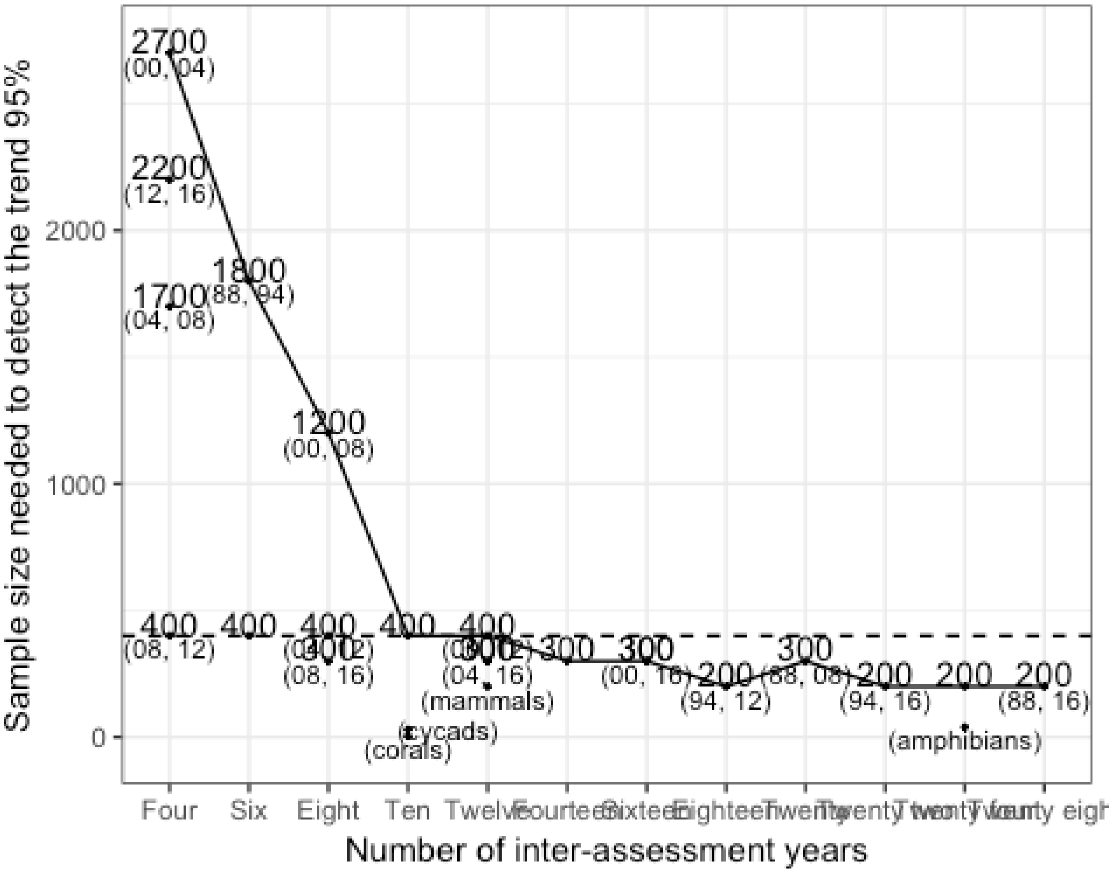


Figure 2.3

Combining this analysis into a single graph, as well as including non-avian groups according to their interassessment length produces Figure 2.3

```
thresholds <- ddply(inter_results, .(n_ass, label), summarize, threshold
= samp_size[min(which(perc_wrong <= 5))])
Maxthresholds <- data.frame(rbindlist(by(thresholds, thresholds$n_ass,
function(X) X[which.max(X$threshold),])))
mammals <- data.frame(n_ass="Twelve", threshold=200)
amphibians <- data.frame(n_ass="Twenty four", threshold=40)
corals <- data.frame(n_ass="Ten", threshold=4)
cycads <- data.frame(n_ass="Ten",threshold=30)




ggplot(Maxthresholds, aes(x=n_ass, y=threshold)) +
 geom_line(group = 1) +
```

```
 geom_text(data = thresholds, aes(label=threshold), color="black",
nudge_y = 50) +
 geom_text(data = thresholds, aes(label=label), color="black", size = 3,
nudge_y = -50, check_overlap = TRUE) +
 geom_point(data = thresholds, size=0.5, color="black") +
 geom_point(data = mammals, aes(), size=0.5, color="black") +
 geom_text(data = mammals, aes(label='(mammals)'), color="black", size =
3, nudge_y = -50) +
 geom_point(data = amphibians, aes(), size=0.5, color="black") +
 geom_text(data = amphibians, aes(label='(amphibians)'), color="black",
size = 3, nudge_y = -50) +
 geom_point(data = corals, aes(), size=0.5, color="black") +
 geom_text(data = corals, aes(label='(corals)'), color="black", size =
3, nudge_y = -50) +
 geom_point(data = cycads, aes(), size=0.5, color="black") +
 geom_text(data = cycads, aes(label='(cycads)'), color="black", size =
3, nudge_x = 0.5) +
 geom_hline(yintercept=400, linetype="dashed") +
 ylab("Sample size needed to detect the trend 95%") +
 xlab("Number of inter-assessment years") +
 theme_bw()
```



5) Representativity

Replicating Baillie et al's approach to 400 sp. sample instead of the 900 sp. used at the time, including representation of DD and applying to all currently available years we get:

Threat class

Distribution of Extinct, Threatened, Non-Threatened and Data-Deficient for samples of 400 Bird sp.

```
#We extract the number (sum) of each Red List category
p_threat <- function(Threat) {
 EX<-sum(Threat=="EX" | Threat=="E", na.rm = T)
 PE<-sum(Threat=="CR(PE)" | Threat=="CR (PE)", na.rm = T)
 EW<-sum(Threat=="EW", na.rm = T)
 PEW<-sum(Threat=="CR(PEW)", na.rm = T)
 CR<-sum(Threat=="CR", na.rm = T)
 EN<-sum(Threat=="EN", na.rm = T)
```

```
 VU<-sum(Threat=="VU" | Threat=="T", na.rm = T)
 NT<-sum(Threat=="NT" | Threat=="LR/nt", na.rm = T)
 LC<-sum(Threat=="LC" | Threat=="LR/lc" | Threat=="LR/cd", na.rm = T)
 DD<-sum(Threat=="DD")

#Measure the percentage of broader threat category, such as:
#Extinct
 PrEx <- sum(EX, PE, EW, PEW)/length(Threat)
#Threatened
 PrTh <- sum(CR, EN, VU)/length(Threat)
#Non-Threatened
 PrNT <- sum (NT, LC)/length(Threat)
#Data-Deficient
 PrDD <- sum (DD)/length(Threat)

#Placing the data in a data.frame
 df <- data.frame(PrEx, PrTh, PrNT, PrDD)
 return(df)
}

Threat <- read.csv("Bird_RLI.csv")
df = data.frame(Threat$binomial, Threat$taxonid, Threat$X1988,
Threat$X1994, Threat$X2000, Threat$X2004, Threat$X2008, Threat$X2012,
Threat$X2016)

#The percentags for each availble year (as of 2019)
p_threat(Threat$X1988)
p_threat(Threat$X1994)
p_threat(Threat$X2000)
p_threat(Threat$X2004)
p_threat(Threat$X2008)
p_threat(Threat$X2012)
p_threat(Threat$X2016)

#Setting simulation number for samples of 100 species to a maximum of
1000 at increments of 100.
```

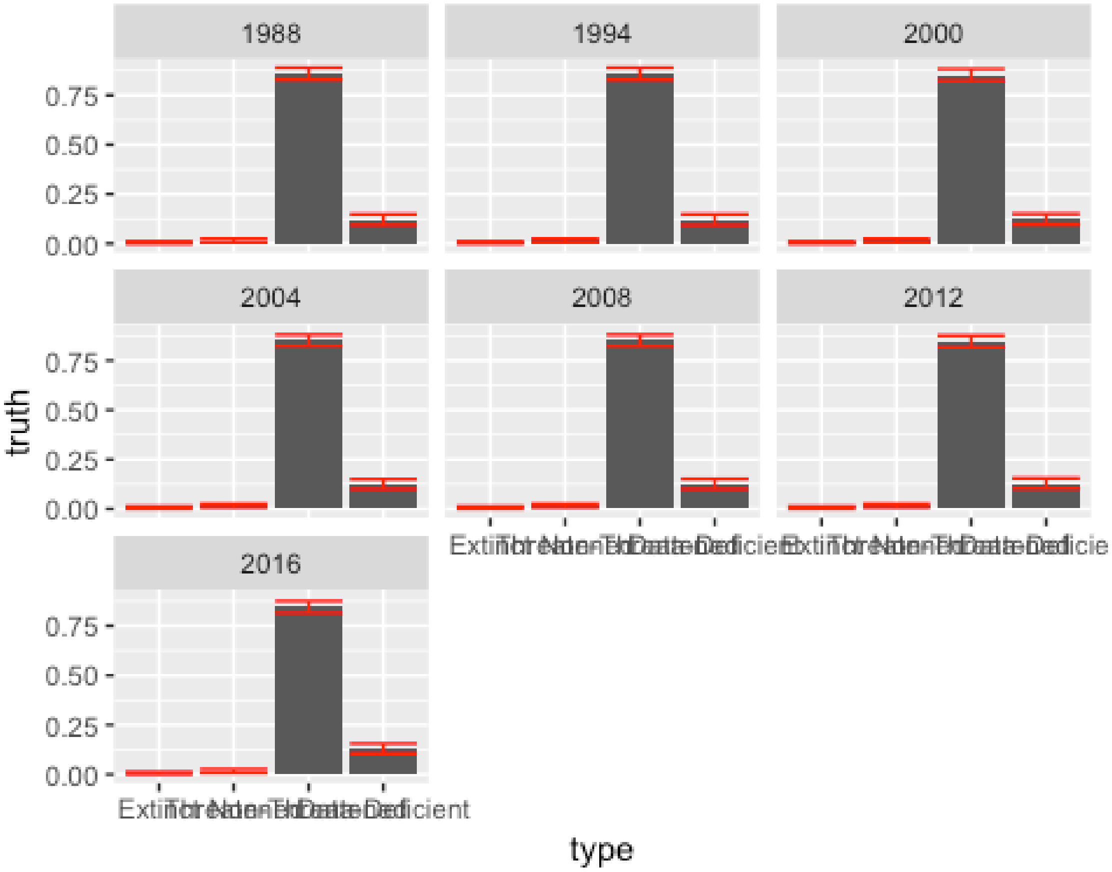
```
sim = 50000
samplist = seq(100, 1000, by=100)
units<-1:length(Threat$binomial)
samp <- nrow(Threat)

# loop to calculate threat percentages of these samples
for (samp in samplist) {
 results = list()
 for (i in 1:sim) {
 random.sample<-sample(units,samp)
 random.birds<-Threat[random.sample,]

 a=p_threat(random.birds$X1988)
 a$year = 1988
 b=p_threat(random.birds$X1994)
 b$year = 1994
 c=p_threat(random.birds$X2000)
 c$year = 2000
 d=p_threat(random.birds$X2004)
 d$year = 2004
 e=p_threat(random.birds$X2008)
 e$year = 2008
 f=p_threat(random.birds$X2012)
 f$year = 2012
 g=p_threat(random.birds$X2016)
 g$year = 2016

 this_result = rbind(a, b, c, d, e, f, g)
 results[[i]] = this_result
 }

 results_df = rbindlist(results)
 # writes out to a file
 write.csv(results_df, file=paste("random_sample_of", samp,
"sp_threat_classes.csv", sep=""))
}
```

Figure S2.7

```
#As mentioned, this analysis used the most updated dataset currently
available updated_Bird_data, under a smaller sample size of 400 sp.
which is consistent with our results.

# For an exact replication of the original results by Baillie et al.,
this analysis can be run with the original data set available at the
time birds<-read.delim("birds_RLIchanges.txt",header=T,as.is=TRUE), with
a sample size of 900 sp. Which wil produce the results and hence the
same exact chart found in the original publication

Threat <- read.csv("Bird_RLI.csv")

#RF: Samp<-400
samp<-400

#data wrangling
df <- read.csv(file=paste("random_sample_of", samp,
"sp_threat_classes.csv", sep=""))
df88 <- subset(df, year==1988)
df94 <- subset(df, year==1994)
df00 <- subset(df, year==2000)
df04 <- subset(df, year==2004)
df08 <- subset(df, year==2008)
df12 <- subset(df, year==2012)
df16 <- subset(df, year==2016)

#creating a calculation of the min&max threat class percentages for each
sample size simulation
get_cis = function(sample) {
 ci_ex = quantile(sample$PrEx, p=c(0.025, 0.975))
 ci_nt = quantile(sample$PrNT, p=c(0.025, 0.975))
 ci_th = quantile(sample$PrTh, p=c(0.025, 0.975))
 ci_dd = quantile(sample$PrDD, p=c(0.025, 0.975))

 lwr = c(ci_ex[1],ci_th[1], ci_nt[1],ci_dd[1])
```

```r
 upr = c(ci_ex[2],ci_th[2], ci_nt[2],ci_dd[2])
 type = c("ex","th", "nt", "dd")

 df = data.frame(type=type, lwr=lwr, upr=upr)
 return(df)
}



#data wrangling per year
Tclasses_1988 <-p_threat(Threat$X1988)
Tclasses_1988 <- t(Tclasses_1988)
cis_88 <- get_cis(df88)
cis_88$truth <- Tclasses_1988[, 1]
cis_88$year <- 1988
Tclasses_1988 <- data.frame(cis_88)

Tclasses_1994 <-p_threat(Threat$X1994)
Tclasses_1994 <- t(Tclasses_1994)
cis_94 <- get_cis(df94)
cis_94$truth <- Tclasses_1994[, 1]
cis_94$year <- 1994
Tclasses_1994 <- data.frame(cis_94)

Tclasses_2000 <-p_threat(Threat$X2000)
Tclasses_2000 <- t(Tclasses_2000)
cis_00 <- get_cis(df00)
cis_00$truth <- Tclasses_2000[, 1]
cis_00$year <- 2000
Tclasses_2000 <- data.frame(cis_00)

Tclasses_2004 <-p_threat(Threat$X2004)
Tclasses_2004 <- t(Tclasses_2004)
cis_04 <- get_cis(df04)
cis_04$truth <- Tclasses_2004[, 1]
cis_04$year <- 2004
Tclasses_2004 <- data.frame(cis_04)
```

```
Tclasses_2008 <-p_threat(Threat$X2008)
Tclasses_2008 <- t(Tclasses_2008)
cis_08 <- get_cis(df08)
cis_08$truth <- Tclasses_2008[, 1]
cis_08$year <- 2008
Tclasses_2008 <- data.frame(cis_08)

Tclasses_2012 <-p_threat(Threat$X2012)
Tclasses_2012 <- t(Tclasses_2012)
cis_12 <- get_cis(df12)
cis_12$truth <- Tclasses_2012[, 1]
cis_12$year <- 2012
Tclasses_2012 <- data.frame(cis_12)

Tclasses_2016 <-p_threat(Threat$X2016)
Tclasses_2016 <- t(Tclasses_2016)
cis_16 <- get_cis(df16)
cis_16$truth <- Tclasses_2016[, 1]
cis_16$year <- 2016
Tclasses_2016 <- data.frame(cis_16)

Tclasses_all <- rbind(Tclasses_1988, Tclasses_1994, Tclasses_2000,
Tclasses_2004, Tclasses_2008, Tclasses_2012, Tclasses_2016)
dodge <- position_dodge(width = 0.9)
names <- c("Extinct", "Threatened", "Non-Threatened", "Data-Deficient")
levels(Tclasses_all$type) <- names

ggplot(data = Tclasses_all)+
 geom_bar(aes(x = type, y = truth), stat="identity")+
 geom_errorbar(aes(type, ymin = lwr, ymax = upr),
 colour = 'red',
 position = dodge)+
 facet_wrap(~year)
```



Biogeographic realms

Distribution of Palearctic, Afrotropical, Indomalayan, Australasian_Oceanic, Nearctic, Neotropical for samples of 400 Bird sp.

```
p_biogeo <- function(Biogeography) {
 p_biog <-sum(Biogeography== 1, na.rm = T)
 #get the percentage of the Environemntal systems
 P_biog <- p_biog/length(Biogeography)
 df <- data.frame(P_biog)
 return(df)
}

realms <- read.csv("ecosystems_realms.csv", as.is=T)


Biogeography = data.frame(Binomial = realms$Binomial,
 Palearctic=realms$Palearctic,
 Afrotropical=realms$Afrotropical,
 Indomalayan=realms$Indomalayan,
```

```
 Australasian.Oceanic=realms$Australasian.Oceanic,
 Nearctic=realms$Nearctic,
 Neotropical=realms$Neotropical)


#having a look at the values for Ecosystems
p_biogeo(Biogeography$Palearctic)
p_biogeo(Biogeography$Afrotropical)
p_biogeo(Biogeography$Indomalayan)
p_biogeo(Biogeography$Australasian.Oceanic)
p_biogeo(Biogeography$Nearctic)
p_biogeo(Biogeography$Neotropical)

###C2)Setting simulation number for randomn sample of 200 and 400
species####
sim = 50000
samplist = seq(100, 1000, by=100)
units<-1:length(Biogeography$Binomial)
samp <- nrow(Biogeography)

# loop to calculate ecosystems percentages
for (samp in samplist) {
 results = list()
 for (i in 1:sim) {
 random.sample<-sample(units,samp)
 random.birds<-Biogeography[random.sample,]

 a=p_biogeo(random.birds$Palearctic)
 a$biogeo = "Palearctic"
 b=p_biogeo(random.birds$Afrotropical)
 b$biogeo = "Afrotropical"
 c=p_biogeo(random.birds$Indomalayan)
 c$biogeo = "Indomalayan"
 d=p_biogeo(random.birds$Australasian.Oceanic)
 d$biogeo = "Australasian.Oceanic"
 e=p_biogeo(random.birds$Nearctic)
```

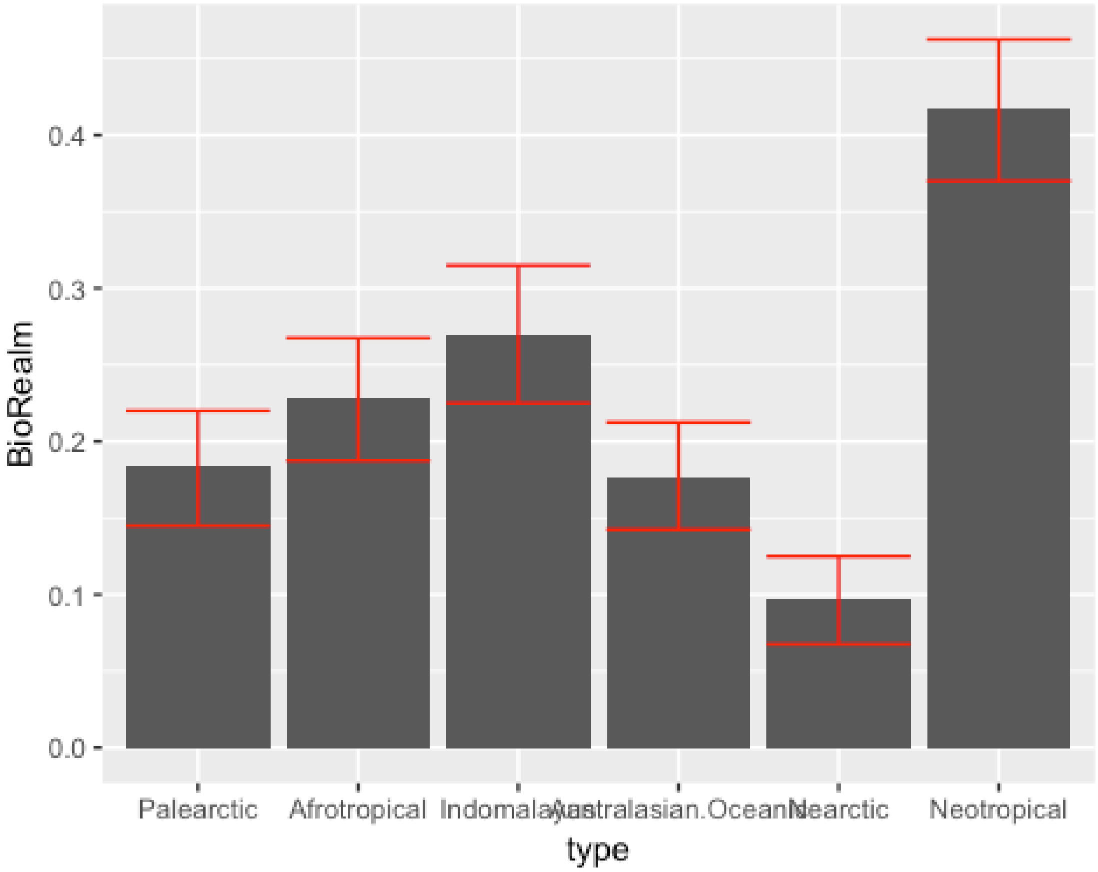
```
 e$biogeo = "Nearctic"
 f=p_biogeo(random.birds$Neotropical)
 f$biogeo = "Neotropical"

 this_result = rbind(a, b, c, d, e, f)
 results[[i]] = this_result
 }

 results_df = rbindlist(results)
 # writes out to a file
 write.csv(results_df, file=paste("random_sample_of", samp,
"sp_biogeographical_realms.csv", sep=""))
}
```

Figure S2.8

```
#Replicating the analysis performed in Baillie et all., we used the
original biogeographical data available for the bird species recognized
at the time under a smaller sample size of 400 sp. which is consistent
with our results.

# For an exact replication of the original results by Baillie et al.,
this analysis can be run for a sample size of 900 sp. Which wil produce
the results and hence the same exact chart found in the original
publication

samp <-400

realms <- read.csv("ecosystems_realms.csv", as.is=T)

Biogeography = data.frame(Binomial = realms$Binomial,
 Palearctic=realms$Palearctic,
 Afrotropical=realms$Afrotropical,
 Indomalayan=realms$Indomalayan,
 Australasian.Oceanic=realms$Australasian.Oceanic,
 Nearctic=realms$Nearctic,
 Neotropical=realms$Neotropical)
```

```
# This can't work, needs a 'paste':
# df <- read.csv("random_sample_of", samp,
"sp_biogeographical_realms.csv", sep="")
df <- read.csv(paste("random_sample_of", samp,
"sp_biogeographical_realms.csv", sep=""))



#calculating the min&max Confidence intervals (CI) values of each
ecosystem
Biogeo_cis = function(sample) {
 ci_Pa <- quantile(subset(sample, biogeo=="Palearctic")$P_biog,
p=c(0.025, 0.975))
 ci_Af <- quantile(subset(sample, biogeo=="Afrotropical")$P_biog,
p=c(0.025, 0.975))
 ci_In <- quantile(subset(sample, biogeo=="Indomalayan")$P_biog,
p=c(0.025, 0.975))
 ci_Au <- quantile(subset(sample,
biogeo=="Australasian.Oceanic")$P_biog, p=c(0.025, 0.975))
 ci_Nea <- quantile(subset(sample, biogeo=="Nearctic")$P_biog,
p=c(0.025, 0.975))
 ci_Neo <- quantile(subset(sample, biogeo=="Neotropical")$P_biog,
p=c(0.025, 0.975))

 lwr = c(ci_Pa[1], ci_Af[1], ci_In[1], ci_Au[1], ci_Nea[1], ci_Neo[1])
 upr = c(ci_Pa[2], ci_Af[2], ci_In[2], ci_Au[2], ci_Nea[2], ci_Neo[2])
 type = c("Palearctic","Afrotropical", "Indomalayan",
"Australasian.Oceanic", "Nearctic","Neotropical")
 df = data.frame(type=type, lwr=lwr, upr=upr)
 return(df)
}

#data wrangling
Palearctic <-p_biogeo(Biogeography$Palearctic)
Afrotropical <-p_biogeo(Biogeography$Afrotropical)
Indomalayan <-p_biogeo(Biogeography$Indomalayan)
```

```
Australasian.Oceanic <-p_biogeo(Biogeography$Australasian.Oceanic)
Nearctic <-p_biogeo(Biogeography$Nearctic)
Neotropical <-p_biogeo(Biogeography$Neotropical)

BioRealm <- data.frame(Palearctic[,1], Afrotropical[,1],
Indomalayan[,1], Australasian.Oceanic[,1], Nearctic[,1],
Neotropical[,1])
BioRealm <- t(BioRealm)
cis <- Biogeo_cis(df)
df_Biogeo <- data.frame(BioRealm, cis)

dodge <- position_dodge(width = 0.9)

names <- c("Palearctic","Afrotropical", "Indomalayan",
"Australasian.Oceanic", "Nearctic","Neotropical")
df_Biogeo$type <-factor(df_Biogeo$type, levels = names)

levels(df_Biogeo$type) <- names



ggplot(data = df_Biogeo)+
 geom_bar(aes(x = type, y = BioRealm), stat="identity")+
 geom_errorbar(aes(type, ymin = lwr, ymax = upr),
 colour = 'red',
 position = dodge)
```



Environments

Distribution of Marine, terrestrial and freshwater environments for samples of 400 Bird sp.

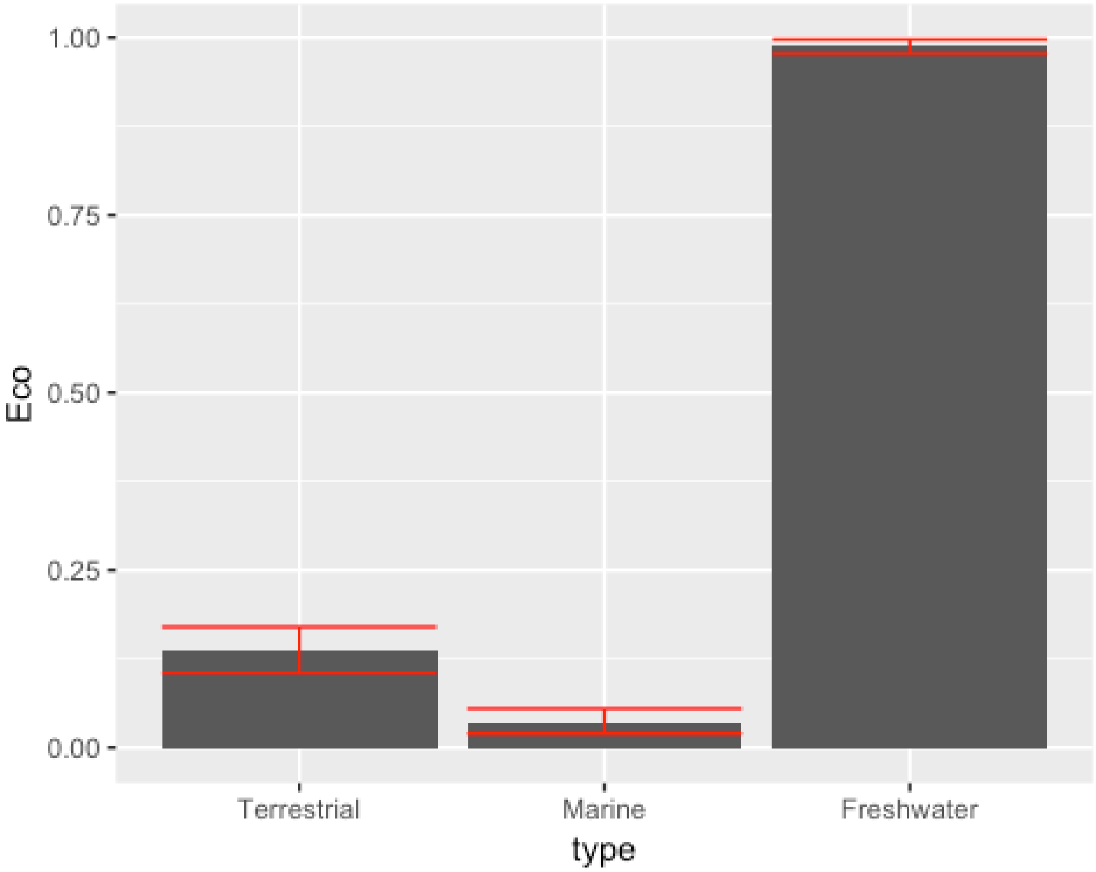
```
p_ecosystems <- function(Ecosystems) {
 p_eco <-sum(Ecosystems== 1, na.rm = T)
 #get the percentage of the Ecosystem
 P_eco <- p_eco/length(Ecosystems)
 df <- data.frame(P_eco)
 return(df)
}

ecosystems_realms <- read.csv("ecosystems_realms.csv", as.is=T)


Ecosystems = data.frame(Binomial = ecosystems_realms$Binomial,
 Terrestrial=ecosystems_realms$Terrestrial,
 Marine=ecosystems_realms$Marine,
 Freshwater=ecosystems_realms$Freshwater)
```

```
#having a look at the values for Ecosystems
p_ecosystems(Ecosystems$Terrestrial)
p_ecosystems(Ecosystems$Marine)
p_ecosystems(Ecosystems$Freshwater)

###B2)Setting simulation number for randomn sample of 400 species####
sim = 50000
samplist = seq(100, 1000, by=100)
units<-1:length(Ecosystems$Binomial)
samp <- nrow(Ecosystems)

# loop to calculate ecosystems percentages
for (samp in samplist) {
 results = list()
 for (i in 1:sim) {
 random.sample<-sample(units,samp)
 random.birds<-Ecosystems[random.sample,]

 a=p_ecosystems(random.birds$Terrestrial)
 a$eco = "Terrestrial"
 b=p_ecosystems(random.birds$Marine)
 b$eco = "Marine"
 c=p_ecosystems(random.birds$Freshwater)
 c$eco = "Freshwater"

 this_result = rbind(a, b, c)
 results[[i]] = this_result
}

results_df = rbindlist(results)
# writes out to a file
write.csv(results_df, file=paste("random_sample_of", samp,
"sp_ecosystems.csv", sep=""))
}
```

Figure S2.9

```
#Replicating the analysis performed in Baillie et all., we used the original environments data available for the bird species recognized at the time under a smaller sample size of 400 sp. which is consistent with our results.

# For an exact replication of the original results by Baillie et al., this analysis can be run for a sample size of 900 sp. Which wil produce the results and hence the same exact chart found in the original publication

#Samp <-400
samp <-400

df <- read.csv(file=paste("random_sample_of", samp,"sp_ecosystems.csv", sep=""))

ecosystems_realms <- read.csv("ecosystems_realms.csv", as.is=T)

Ecosystems = data.frame(Binomial = ecosystems_realms$Binomial,
 Terrestrial=ecosystems_realms$Terrestrial,
 Marine=ecosystems_realms$Marine,
 Freshwater=ecosystems_realms$Freshwater)
#calculating the min&max values of each ecosystem
Eco_cis = function(sample) {
 ci_Ter <- quantile(subset(sample, eco=="Terrestrial")$P_eco, p=c(0.025, 0.975))
 ci_Mar <- quantile(subset(sample, eco=="Marine")$P_eco, p=c(0.025, 0.975))
 ci_Fw <- quantile(subset(sample, eco=="Freshwater")$P_eco, p=c(0.025, 0.975))

 lwr = c(ci_Ter[1], ci_Mar[1], ci_Fw[1])
 upr = c(ci_Ter[2], ci_Mar[2], ci_Fw[2])
 type = c("Terrestrial","Marine", "Freshwater")
 df = data.frame(type=type, lwr=lwr, upr=upr)
```

```
 return(df)
}

#Some data wrangling
Terrestrial <-p_ecosystems(Ecosystems$Terrestrial)
Marine <-p_ecosystems(Ecosystems$Marine)
Freshwater <-p_ecosystems(Ecosystems$Freshwater)
Eco <- data.frame(Terrestrial[,1], Marine[,1], Freshwater[,1])
Eco <- t(Eco)
cis <- Eco_cis(df)
df_Ecosystems <- data.frame(Eco, cis)

dodge <- position_dodge(width = 0.9)

names <- c("Terrestrial", "Marine", "Freshwater")
levels(df_Ecosystems$type) <- names

ggplot(data = df_Ecosystems)+
 geom_bar(aes(x = type, y = Eco), stat="identity")+
 geom_errorbar(aes(type, ymin = lwr, ymax = upr),
 colour = 'red',
 position = dodge)
```



Taxonomy

Distribution of taxonomic orders for samples of 400 Bird sp.

```
#For this analysis of Bird Taxonomic orders we used the most updatabase: HBW and BirdLife International (2017) Handbook of the Birds of the World and BirdLife International digital checklist of the birds of the world. Version 2 Available at: http://datazone.birdlife.org/userfiles/file/Species/Taxonomy/HBW-BirdLife_Checklist_Version_2.zip [.xls zipped 1 MB]. For more details, see: http://datazone.birdlife.org/species/taxonomy"

# For an exact replication of the original results by Baillie et al., this analysis can be run for the file birds<-read.delim("birds_RLIchanges.txt",header=T,as.is=TRUE). Which wil produce the results and hence the same exact chart found in the original publication
```

```
Bird_data <- read.csv("HBW-BirdLife_Checklist_Version_2b.csv")

BOrders = data.frame(Species = Bird_data$Scientific.name,
 Orders = Bird_data$Order)


#get the number (%) of each order
p_order <- function(Order) {
 #percentage of each order
 ACCIPITRIFORMES <- sum (Order=="ACCIPITRIFORMES")/length(Order)
 ANSERIFORMES <- sum (Order=="ANSERIFORMES")/length(Order)
 BUCEROTIFORMES <- sum (Order=="BUCEROTIFORMES")/length(Order)
 CAPRIMULGIFORMES <- sum (Order=="CAPRIMULGIFORMES")/length(Order)
 CARIAMIFORMES <- sum (Order=="CARIAMIFORMES")/length(Order)
 CATHARTIFORMES <- sum (Order=="CATHARTIFORMES")/length(Order)
 CHARADRIIFORMES <- sum (Order=="CHARADRIIFORMES")/length(Order)
 CICONIIFORMES <- sum (Order=="CICONIIFORMES")/length(Order)
 COLIIFORMES <- sum (Order=="COLIIFORMES")/length(Order)
 COLUMBIFORMES <- sum (Order=="COLUMBIFORMES")/length(Order)
 CORACIIFORMES <- sum (Order=="CORACIIFORMES")/length(Order)
 CUCULIFORMES <- sum (Order=="CUCULIFORMES")/length(Order)
 EURYPYGIFORMES <- sum (Order=="EURYPYGIFORMES")/length(Order)
 FALCONIFORMES <- sum (Order=="FALCONIFORMES")/length(Order)
 GALLIFORMES <- sum (Order=="GALLIFORMES")/length(Order)
 GAVIIFORMES <- sum (Order=="GAVIIFORMES")/length(Order)
 GRUIFORMES <- sum (Order=="GRUIFORMES")/length(Order)
 LEPTOSOMIFORMES <- sum (Order=="LEPTOSOMIFORMES")/length(Order)
 MESITORNITHIFORMES <- sum (Order=="MESITORNITHIFORMES")/length(Order)
 MUSOPHAGIFORMES <- sum (Order=="MUSOPHAGIFORMES")/length(Order)
 OPISTHOCOMIFORMES <- sum (Order=="OPISTHOCOMIFORMES")/length(Order)
 OTIDIFORMES <- sum (Order=="OTIDIFORMES")/length(Order)
 PASSERIFORMES <- sum (Order=="PASSERIFORMES")/length(Order)
 PELECANIFORMES <- sum (Order=="PELECANIFORMES")/length(Order)
 PHAETHONTIFORMES <- sum (Order=="PHAETHONTIFORMES")/length(Order)
 PHOENICOPTERIFORMES <- sum (Order=="PHOENICOPTERIFORMES")/length(Order)
 PICIFORMES <- sum (Order=="PICIFORMES")/length(Order)
```

```
 PODICIPEDIFORMES <- sum (Order=="PODICIPEDIFORMES")/length(Order)
 PROCELLARIIFORMES <- sum (Order=="PROCELLARIIFORMES")/length(Order)
 PSITTACIFORMES <- sum (Order=="PSITTACIFORMES")/length(Order)
 PTEROCLIFORMES <- sum (Order=="PTEROCLIFORMES")/length(Order)
 SPHENISCIFORMES <- sum (Order=="SPHENISCIFORMES")/length(Order)
 STRIGIFORMES <- sum (Order=="STRIGIFORMES")/length(Order)
 STRUTHIONIFORMES <- sum (Order=="STRUTHIONIFORMES")/length(Order)
 SULIFORMES <- sum (Order=="SULIFORMES")/length(Order)
 TROGONIFORMES <- sum (Order=="TROGONIFORMES")/length(Order)

 #Place the data in a data.frame
 df <- data.frame(PASSERIFORMES, CHARADRIIFORMES, ACCIPITRIFORMES,
ANSERIFORMES,
 PELECANIFORMES, CAPRIMULGIFORMES, PICIFORMES, PROCELLARIIFORMES,
 COLUMBIFORMES, GRUIFORMES, STRIGIFORMES, CORACIIFORMES,
 GALLIFORMES, FALCONIFORMES, CUCULIFORMES, PSITTACIFORMES,
 SULIFORMES, BUCEROTIFORMES, CICONIIFORMES, PODICIPEDIFORMES,
 OTIDIFORMES, PTEROCLIFORMES, STRUTHIONIFORMES, TROGONIFORMES,
 PHAETHONTIFORMES, GAVIIFORMES, MUSOPHAGIFORMES, PHOENICOPTERIFORMES,
 CATHARTIFORMES, SPHENISCIFORMES, COLIIFORMES, EURYPYGIFORMES,
 OPISTHOCOMIFORMES, CARIAMIFORMES, LEPTOSOMIFORMES, MESITORNITHIFORMES)
 return(df)
}


#having a look at the values for each year
Orders_p <- p_order(BOrders$Orders)

#D2)Setting simulation number for randomn sample of 400 species####
sim = 50000
samplist = seq(100, 1000, by=100)
units<-1:length(BOrders$Orders)
samp <- nrow(BOrders)

# loop to calculate order percentage
for (samp in samplist) {
```

```
 results = list()
 for (i in 1:sim) {
 random.sample<-sample(units,samp)
 random.birds<-BOrders[random.sample,]

 a=p_order(random.birds$Orders)

 this_result = rbind(a)
 results[[i]] = this_result
 }

 results_df = rbindlist(results)

 #If usefull for future analysis a column can be added with how many
orders are not represented in each sample size
 #results_df$missed <- rowSums(results_df == 0)

 # writes out to a file
 write.csv(results_df, file=paste("random_sample_of", samp,
"sp_order.csv", sep=""))
}
```

Figure S2.10

```
Bird_data <- read.csv("HBW-BirdLife_Checklist_Version_2b.csv")

BOrders = data.frame(Species = Bird_data$Scientific.name,
 Orders = Bird_data$Order)

#get the number (%) of each order
p_order <- function(Order) {
 #percentage of each order
 ACCIPITRIFORMES <- sum (Order=="ACCIPITRIFORMES")/length(Order)
 ANSERIFORMES <- sum (Order=="ANSERIFORMES")/length(Order)
 BUCEROTIFORMES <- sum (Order=="BUCEROTIFORMES")/length(Order)
 CAPRIMULGIFORMES <- sum (Order=="CAPRIMULGIFORMES")/length(Order)
 CARIAMIFORMES <- sum (Order=="CARIAMIFORMES")/length(Order)
```

```
CATHARTIFORMES <- sum (Order=="CATHARTIFORMES")/length(Order)
CHARADRIIFORMES <- sum (Order=="CHARADRIIFORMES")/length(Order)
CICONIIFORMES <- sum (Order=="CICONIIFORMES")/length(Order)
COLIIFORMES <- sum (Order=="COLIIFORMES")/length(Order)
COLUMBIFORMES <- sum (Order=="COLUMBIFORMES")/length(Order)
CORACIIFORMES <- sum (Order=="CORACIIFORMES")/length(Order)
CUCULIFORMES <- sum (Order=="CUCULIFORMES")/length(Order)
EURYPYGIFORMES <- sum (Order=="EURYPYGIFORMES")/length(Order)
FALCONIFORMES <- sum (Order=="FALCONIFORMES")/length(Order)
GALLIFORMES <- sum (Order=="GALLIFORMES")/length(Order)
GAVIIFORMES <- sum (Order=="GAVIIFORMES")/length(Order)
GRUIFORMES <- sum (Order=="GRUIFORMES")/length(Order)
LEPTOSOMIFORMES <- sum (Order=="LEPTOSOMIFORMES")/length(Order)
MESITORNITHIFORMES <- sum (Order=="MESITORNITHIFORMES")/length(Order)
MUSOPHAGIFORMES <- sum (Order=="MUSOPHAGIFORMES")/length(Order)
OPISTHOCOMIFORMES <- sum (Order=="OPISTHOCOMIFORMES")/length(Order)
OTIDIFORMES <- sum (Order=="OTIDIFORMES")/length(Order)
PASSERIFORMES <- sum (Order=="PASSERIFORMES")/length(Order)
PELECANIFORMES <- sum (Order=="PELECANIFORMES")/length(Order)
PHAETHONTIFORMES <- sum (Order=="PHAETHONTIFORMES")/length(Order)
PHOENICOPTERIFORMES <- sum (Order=="PHOENICOPTERIFORMES")/length(Order)
PICIFORMES <- sum (Order=="PICIFORMES")/length(Order)
PODICIPEDIFORMES <- sum (Order=="PODICIPEDIFORMES")/length(Order)
PROCELLARIIFORMES <- sum (Order=="PROCELLARIIFORMES")/length(Order)
PSITTACIFORMES <- sum (Order=="PSITTACIFORMES")/length(Order)
PTEROCLIFORMES <- sum (Order=="PTEROCLIFORMES")/length(Order)
SPHENISCIFORMES <- sum (Order=="SPHENISCIFORMES")/length(Order)
STRIGIFORMES <- sum (Order=="STRIGIFORMES")/length(Order)
STRUTHIONIFORMES <- sum (Order=="STRUTHIONIFORMES")/length(Order)
SULIFORMES <- sum (Order=="SULIFORMES")/length(Order)
TROGONIFORMES <- sum (Order=="TROGONIFORMES")/length(Order)

#Place the data in a data.frame
df <- data.frame(PASSERIFORMES, CHARADRIIFORMES, ACCIPITRIFORMES,
ANSERIFORMES,
PELECANIFORMES, CAPRIMULGIFORMES, PICIFORMES, PROCELLARIIFORMES,
```

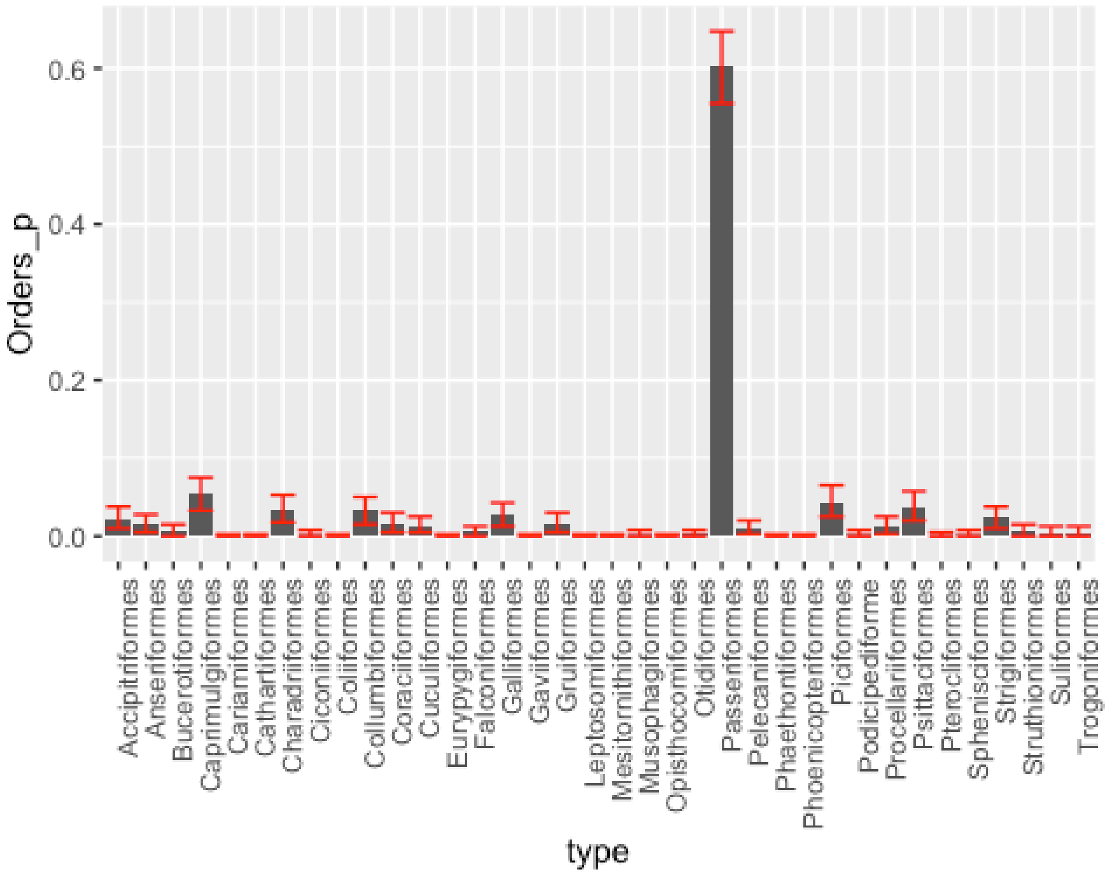
```
 COLUMBIFORMES, GRUIFORMES, STRIGIFORMES, CORACIIFORMES,
 GALLIFORMES, FALCONIFORMES, CUCULIFORMES, PSITTACIFORMES,
 SULIFORMES, BUCEROTIFORMES, CICONIIFORMES, PODICIPEDIFORMES,
 OTIDIFORMES, PTEROCLIFORMES, STRUTHIONIFORMES, TROGONIFORMES,
 PHAETHONTIFORMES, GAVIIFORMES, MUSOPHAGIFORMES, PHOENICOPTERIFORMES,
 CATHARTIFORMES, SPHENISCIFORMES, COLIIFORMES, EURYPYGIFORMES,
 OPISTHOCOMIFORMES, CARIAMIFORMES, LEPTOSOMIFORMES, MESITORNITHIFORMES)
 return(df)
}



#having a look at the values for each year
Orders_p <- p_order(BOrders$Orders)



#The sample size used in the publication is set for 400, but can easily
be changed for 200 or another value here
samp <-400

df_order <- read.csv(file=paste("random_sample_of", samp,
"sp_order.csv", sep=""))

#calculating the min&max values of the sample sizes
order_cis = function(sample) {
 ci_PASSERIFORMES = quantile(sample$PASSERIFORMES, p=c(0.025, 0.975))
 ci_CHARADRIIFORMES =quantile(sample$CHARADRIIFORMES , p=c(0.025,
0.975))
 ci_ACCIPITRIFORMES =quantile(sample$ACCIPITRIFORMES , p=c(0.025,
0.975))
 ci_ANSERIFORMES =quantile(sample$ANSERIFORMES, p=c(0.025, 0.975))
 ci_PELECANIFORMES =quantile(sample$PELECANIFORMES, p=c(0.025, 0.975))
 ci_CAPRIMULGIFORMES =quantile(sample$CAPRIMULGIFORMES, p=c(0.025,
0.975))
 ci_PICIFORMES =quantile(sample$PICIFORMES, p=c(0.025, 0.975))
 ci_PROCELLARIIFORMES =quantile(sample$PROCELLARIIFORMES, p=c(0.025,
0.975))
```

```
 ci_COLUMBIFORMES =quantile(sample$COLUMBIFORMES, p=c(0.025, 0.975))
 ci_GRUIFORMES =quantile(sample$GRUIFORMES, p=c(0.025, 0.975))
 ci_STRIGIFORMES =quantile(sample$STRIGIFORMES, p=c(0.025, 0.975))
 ci_CORACIIFORMES =quantile(sample$CORACIIFORMES, p=c(0.025, 0.975))
 ci_GALLIFORMES =quantile(sample$GALLIFORMES, p=c(0.025, 0.975))
 ci_FALCONIFORMES =quantile(sample$FALCONIFORMES, p=c(0.025, 0.975))
 ci_CUCULIFORMES =quantile(sample$CUCULIFORMES, p=c(0.025, 0.975))
 ci_PSITTACIFORMES =quantile(sample$PSITTACIFORMES, p=c(0.025, 0.975))
 ci_SULIFORMES =quantile(sample$SULIFORMES, p=c(0.025, 0.975))
 ci_BUCEROTIFORMES =quantile(sample$BUCEROTIFORMES, p=c(0.025, 0.975))
 ci_CICONIIFORMES =quantile(sample$CICONIIFORMES, p=c(0.025, 0.975))
 ci_PODICIPEDIFORMES =quantile(sample$PODICIPEDIFORMES, p=c(0.025, 0.975))
 ci_OTIDIFORMES =quantile(sample$OTIDIFORMES, p=c(0.025, 0.975))
 ci_PTEROCLIFORMES =quantile(sample$PTEROCLIFORMES, p=c(0.025, 0.975))
 ci_STRUTHIONIFORMES =quantile(sample$STRUTHIONIFORMES, p=c(0.025, 0.975))
 ci_TROGONIFORMES =quantile(sample$TROGONIFORMES, p=c(0.025, 0.975))
 ci_PHAETHONTIFORMES =quantile(sample$PHAETHONTIFORMES, p=c(0.025, 0.975))
 ci_GAVIIFORMES =quantile(sample$GAVIIFORMES , p=c(0.025, 0.975))
 ci_MUSOPHAGIFORMES =quantile(sample$MUSOPHAGIFORMES, p=c(0.025, 0.975))
 ci_PHOENICOPTERIFORMES =quantile(sample$PHOENICOPTERIFORMES, p=c(0.025, 0.975))
 ci_CATHARTIFORMES =quantile(sample$CATHARTIFORMES, p=c(0.025, 0.975))
 ci_SPHENISCIFORMES =quantile(sample$SPHENISCIFORMES, p=c(0.025, 0.975))
 ci_COLIIFORMES =quantile(sample$COLIIFORMES, p=c(0.025, 0.975))
 ci_EURYPYGIFORMES =quantile(sample$EURYPYGIFORMES, p=c(0.025, 0.975))
 ci_OPISTHOCOMIFORMES =quantile(sample$OPISTHOCOMIFORMES, p=c(0.025, 0.975))
 ci_CARIAMIFORMES =quantile(sample$CARIAMIFORMES, p=c(0.025, 0.975))
 ci_LEPTOSOMIFORMES =quantile(sample$LEPTOSOMIFORMES, p=c(0.025, 0.975))
 ci_MESITORNITHIFORMES =quantile(sample$MESITORNITHIFORMES, p=c(0.025, 0.975))
 lwr=c(
 ci_PASSERIFORMES[1],
```

```
ci_CHARADRIIFORMES[1],
ci_ACCIPITRIFORMES[1],
ci_ANSERIFORMES[1],
ci_PELECANIFORMES[1],
ci_CAPRIMULGIFORMES[1],
ci_PICIFORMES[1],
ci_PROCELLARIIFORMES[1],
ci_COLUMBIFORMES[1],
ci_GRUIFORMES[1],
ci_STRIGIFORMES[1],
ci_CORACIIFORMES[1],
ci_GALLIFORMES[1],
ci_FALCONIFORMES[1],
ci_CUCULIFORMES[1],
ci_PSITTACIFORMES[1],
ci_SULIFORMES[1],
ci_BUCEROTIFORMES[1],
ci_CICONIIFORMES[1],
ci_PODICIPEDIFORMES[1],
ci_OTIDIFORMES[1],
ci_PTEROCLIFORMES[1],
ci_STRUTHIONIFORMES[1],
ci_TROGONIFORMES[1],
ci_PHAETHONTIFORMES[1],
ci_GAVIIFORMES[1],
ci_MUSOPHAGIFORMES[1],
ci_PHOENICOPTERIFORMES[1],
ci_CATHARTIFORMES[1],
ci_SPHENISCIFORMES[1],
ci_COLIIFORMES[1],
ci_EURYPYGIFORMES[1],
ci_OPISTHOCOMIFORMES[1],
ci_CARIAMIFORMES[1],
ci_LEPTOSOMIFORMES[1],
ci_MESITORNITHIFORMES[1]
)
```

```
upr = c(
ci_PASSERIFORMES[2],
ci_CHARADRIIFORMES[2],
ci_ACCIPITRIFORMES[2],
ci_ANSERIFORMES[2],
ci_PELECANIFORMES[2],
ci_CAPRIMULGIFORMES[2],
ci_PICIFORMES[2],
ci_PROCELLARIIFORMES[2],
ci_COLUMBIFORMES[2],
ci_GRUIFORMES[2],
ci_STRIGIFORMES[2],
ci_CORACIIFORMES[2],
ci_GALLIFORMES[2],
ci_FALCONIFORMES[2],
ci_CUCULIFORMES[2],
ci_PSITTACIFORMES[2],
ci_SULIFORMES[2],
ci_BUCEROTIFORMES[2],
ci_CICONIIFORMES[2],
ci_PODICIPEDIFORMES[2],
ci_OTIDIFORMES[2],
ci_PTEROCLIFORMES[2],
ci_STRUTHIONIFORMES[2],
ci_TROGONIFORMES[2],
ci_PHAETHONTIFORMES[2],
ci_GAVIIFORMES[2],
ci_MUSOPHAGIFORMES[2],
ci_PHOENICOPTERIFORMES[2],
ci_CATHARTIFORMES[2],
ci_SPHENISCIFORMES[2],
ci_COLIIFORMES[2],
ci_EURYPYGIFORMES[2],
ci_OPISTHOCOMIFORMES[2],
ci_CARIAMIFORMES[2],
ci_LEPTOSOMIFORMES[2],
```

```
 ci_MESITORNITHIFORMES[2]
 )
 type = c("Passeriformes", "Charadriiformes", "Accipitriformes",
"Anseriformes",
 "Pelecaniformes", "Caprimulgiformes", "Piciformes",
"Procellariiformes",
 "Collumbiformes", "Gruiformes", "Strigiformes", "Coraciiformes",
 "Galliformes", "Falconiformes", "Cuculiformes", "Psittaciformes",
 "Suliformes", "Bucerotiformes", "Ciconiiformes", "Podicipediforme",
 "Otidiformes", "Pterocliformes", "Struthioniformes", "Trogoniformes",
 "Phaethontiformes", "Gaviiformes", "Musophagiformes",
"Phoenicopteriformes",
 "Cathartiformes", "Sphenisciformes", "Coliiformes", "Eurypygiformes",
 "Opisthocomiformes", "Cariamiformes", "Leptosomiformes",
"Mesitornithiformes")

 df = data.frame(type=type, lwr=lwr, upr=upr)
 return(df)
}



#Barplot the data for 2016
#Some data wrangling
Orders_p <- t(Orders_p)
Orders_cis <- order_cis(df_order)
Orders<- data.frame(Orders_p, Orders_cis)



dodge <- position_dodge(width = 0.9)

ggplot(data = Orders)+
 geom_bar(aes(x = type, y = Orders_p), stat="identity")+
 geom_errorbar(aes(type, ymin = lwr, ymax = upr),
 colour = 'red',
 position = dodge)+
 theme(axis.text.x = element_text(angle = 90, hjust = 1))
```



6) Measuring representativity

Measuring the representation of different relevant features of birds, mammals, amphibians, corals and cycads with a chi-squared test

Birds

Using the original data available for the bird species recognized at the time of the original Baillie et all. analysis

```
bird_data <- read.csv("ecosystems_realms.csv")


#testing Environmental Systems
b_systems = bird_data[, c(4:6)]
b_systems_count = colSums(b_systems, na.rm = T)
#testing biogeographical realms
b_realms = bird_data[, c(7:12)]
b_realms_count = colSums(b_realms, na.rm = T)
#testing taxonomical orders
```

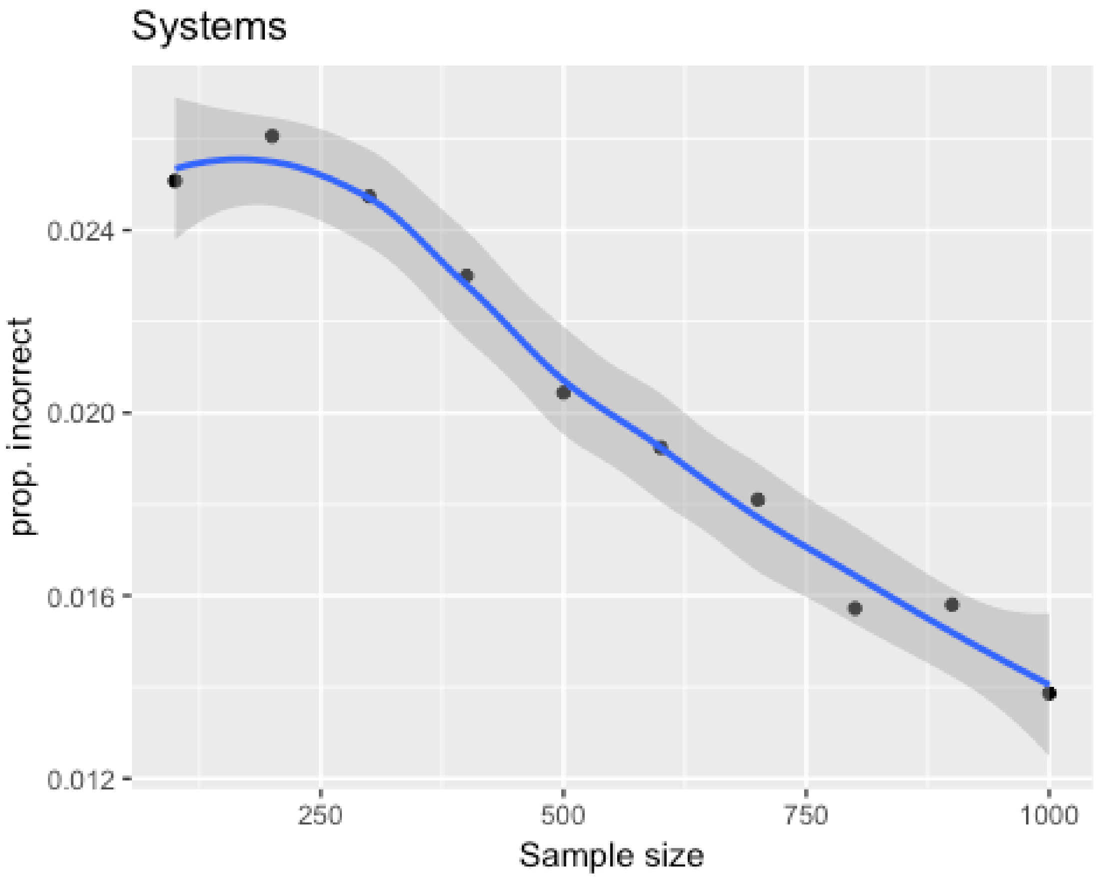
```
b_orders = bird_data$Order
b_order_count = table(b_orders)

# For 50000 replicates
N_REP = 50000
#Setting threshold, sample size and number of replicates
thresh = 0.05
samps = c(seq(100, 1000, by=100))

b_results_system = rep(NA, length(samps))
b_results_realm = rep(NA, length(samps))
b_results_order = rep(NA, length(samps))



for (j in 1:length(samps)) {
 SAMP_SIZE = samps[j]
 cat(sprintf("Sample size %d\n", SAMP_SIZE))

 b_k_system = 0
 b_k_realm = 0
 b_k_order = 0

 for (i in 1:N_REP) {

 bird_sample_idx = sample(1:nrow(bird_data), size = SAMP_SIZE)
 bird_sample_data = bird_data[bird_sample_idx, ]

 # Count how many of each environmental system
 b_systems_sample = bird_sample_data[, c(4:6)]
 b_systems_sample_count = colSums(b_systems_sample, na.rm = T)

 # Count how many of each biogeographi realms
 b_realms_sample = bird_sample_data[, c(7:12)]
 b_realms_sample_count = colSums(b_realms_sample, na.rm = T)
```

```
 # Count how many of each taxonomical order
 b_sample_orders = bird_sample_data$Order
 b_sample_order_count = table(b_sample_orders)

 # Compare these counts
 b_t_system = chisq.test(rbind(b_systems_count, b_systems_sample_count),
simulate.p.value = T)
 b_t_realm = chisq.test(rbind(b_realms_count, b_realms_sample_count),
simulate.p.value = T)
 b_t_order = chisq.test(rbind(b_order_count, b_sample_order_count),
simulate.p.value = T)

 # Record if the difference is significant
 b_p_system = b_t_system$p.value
 b_p_realm = b_t_realm$p.value
 b_p_order = b_t_order$p.value
 if (b_p_system <= thresh) b_k_system = b_k_system + 1
 if (b_p_realm <= thresh) b_k_realm = b_k_realm + 1
 if (b_p_order <= thresh) b_k_order = b_k_order + 1
 }
 # Store frequency of how many were significant.
 b_results_system[j] = b_k_system
 b_results_realm[j] = b_k_realm
 b_results_order[j] = b_k_order

}



b_results_system_df = data.frame(sample_size=samps, k =
b_results_system, N_REP=N_REP)
write.csv(b_results_system_df, file=paste("bird_system.csv"))

b_results_realm_df = data.frame(sample_size=samps, k = b_results_realm,
N_REP=N_REP)
write.csv(b_results_realm_df, file=paste("bird_realms.csv"))
```

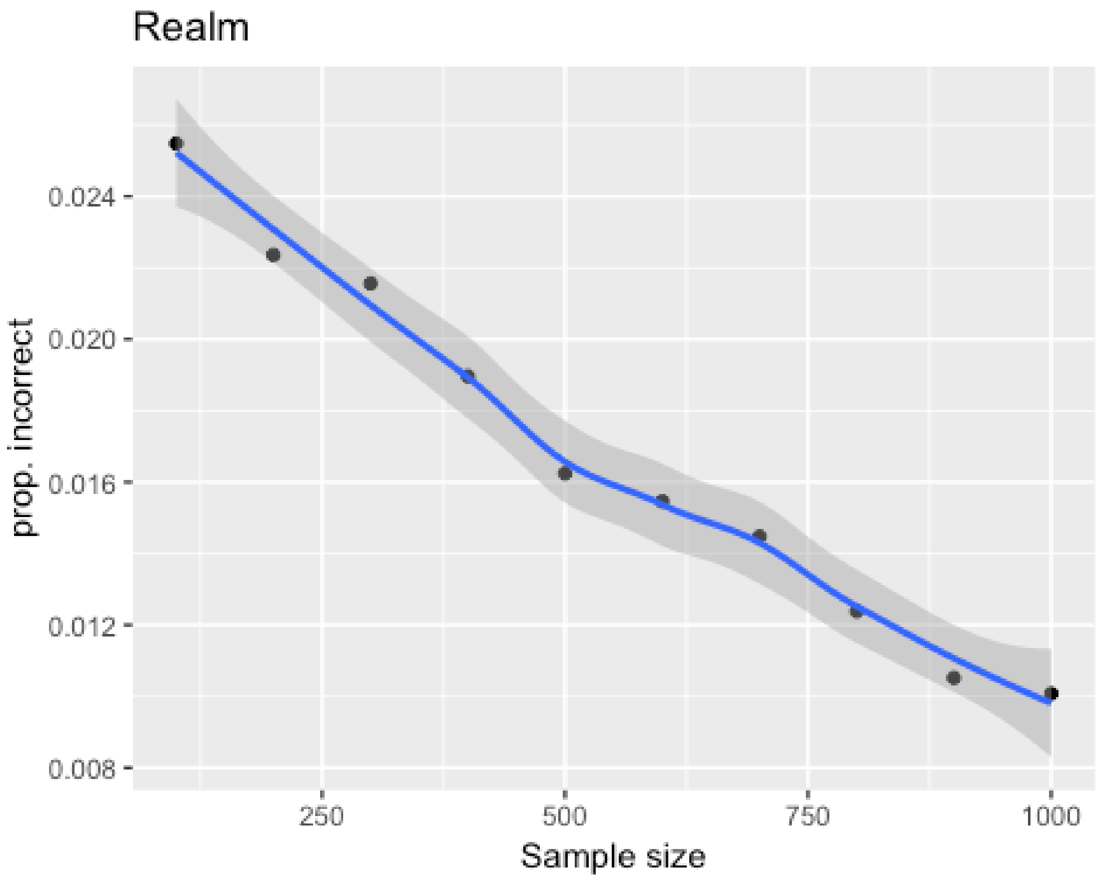
```
b_results_order_df = data.frame(sample_size=samps, k = b_results_order,
N_REP=N_REP)
write.csv(b_results_order_df, file=paste("bird_taxon.csv"))
```

We can then visualise the percentage of random samples with a p value bellow 0.05 for a set of sample sizes

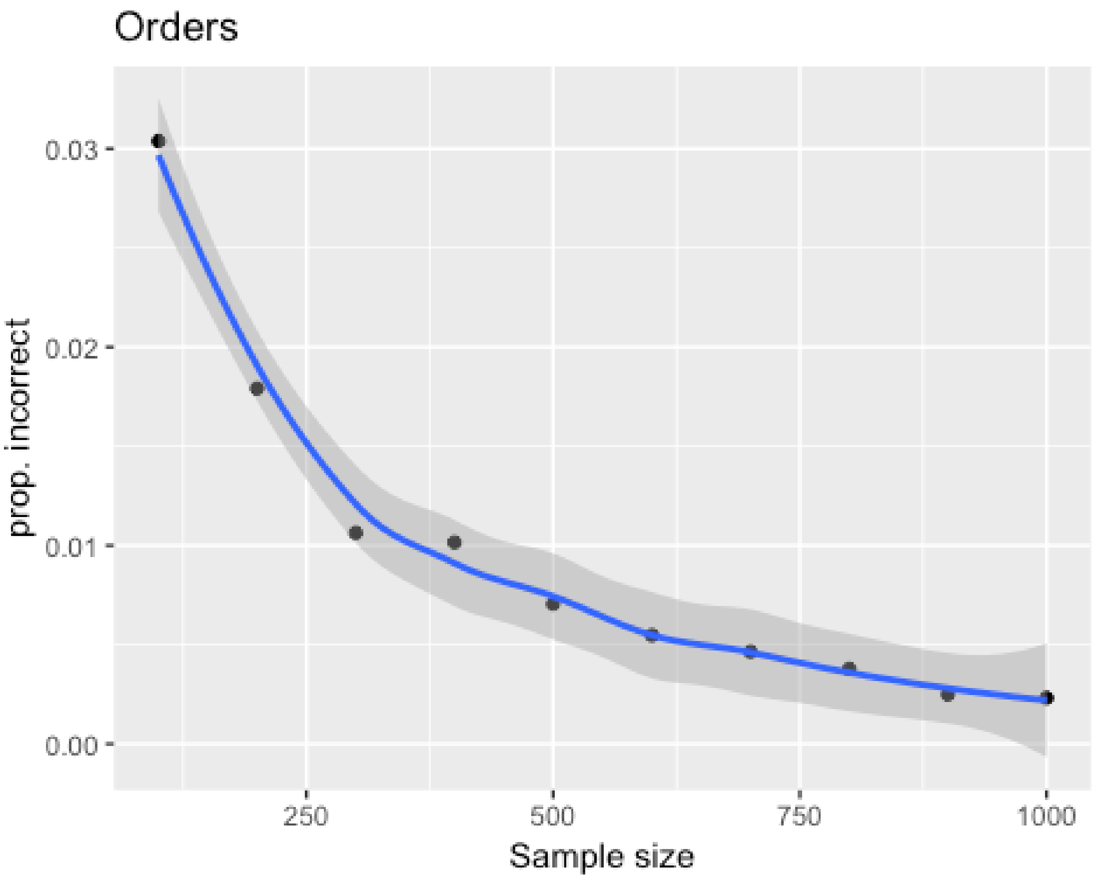
```
bird_system <- read.csv("bird_system.csv")
ggplot(bird_system, aes(x=sample_size, y=(k/N_REP))) + geom_point() +
geom_smooth() + ggtitle("Systems") + xlab("Sample size") + ylab("prop.
incorrect")
```



```
bird_realms <- read.csv("bird_realms.csv")
ggplot(bird_realms, aes(x=sample_size, y=(k/N_REP))) + geom_point() +
geom_smooth() + ggtitle("Realm") + xlab("Sample size") + ylab("prop.
incorrect")
```



```
bird_taxon <- read.csv("bird_taxon.csv")
ggplot(bird_taxon, aes(x=sample_size, y=(k/N_REP))) + geom_point() +
geom_smooth() + ggtitle("Orders") + xlab("Sample size") + ylab("prop.
incorrect")
```



Mammals

Using the same analysis on mammal data

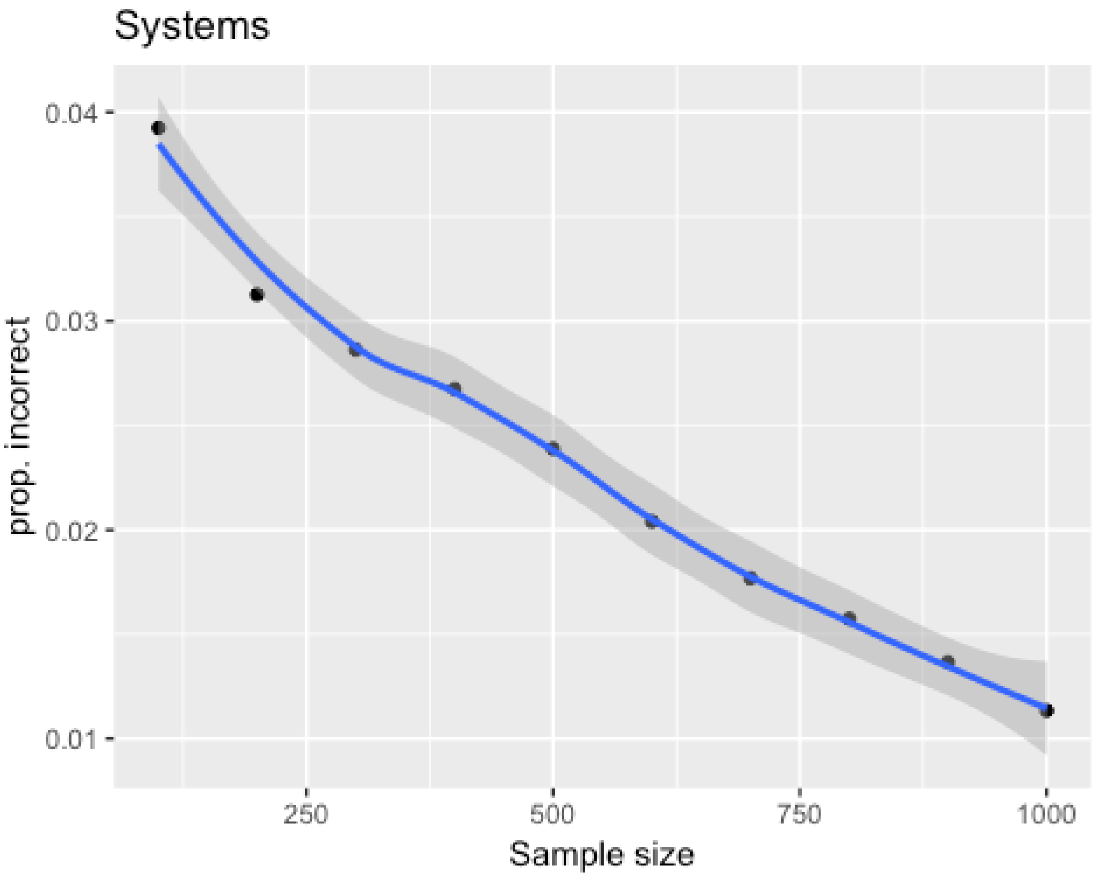
```
mammal_data <- read.csv("Mammal_RLI.csv")

#testing Environmental Systems
m_systems = mammal_data[, c(38:40)]
m_systems_count = colSums(m_systems, na.rm = T)
#testing biogeographical realms
m_realms = mammal_data[, c(30:37)]
m_realms_count = colSums(m_realms, na.rm = T)
#testing taxonomical orders
m_orders = mammal_data$Order
m_order_count = table(m_orders)

# For 50000 replicates
N_REP = 50000
```

```r
#Setting threshold, sample size and number of repetitions
thresh = 0.05
samps = c(seq(100, 1000, by=100))

m_results_system = rep(NA, length(samps))
m_results_realm = rep(NA, length(samps))
m_results_order = rep(NA, length(samps))



for (j in 1:length(samps)) {
 SAMP_SIZE = samps[j]
 cat(sprintf("Sample size %d\n", SAMP_SIZE))

 m_k_system = 0
 m_k_realm = 0
 m_k_order = 0
 # For 1000 replicates
 for (i in 1:N_REP) {

 mam_sample_idx = sample(1:nrow(mammal_data), size = SAMP_SIZE)
 mam_sample_data = mammal_data[mam_sample_idx, ]

 # Count how many of each system
 m_systems_sample = mam_sample_data[, c(38:40)]
 m_systems_sample_count = colSums(m_systems_sample, na.rm = T)

 # Count how many of each system
 m_realms_sample = mam_sample_data[, c(30:37)]
 m_realms_sample_count = colSums(m_realms_sample, na.rm = T)

 # Count how many of each order
 m_sample_orders = mam_sample_data$Order
 m_sample_order_count = table(m_sample_orders)
```

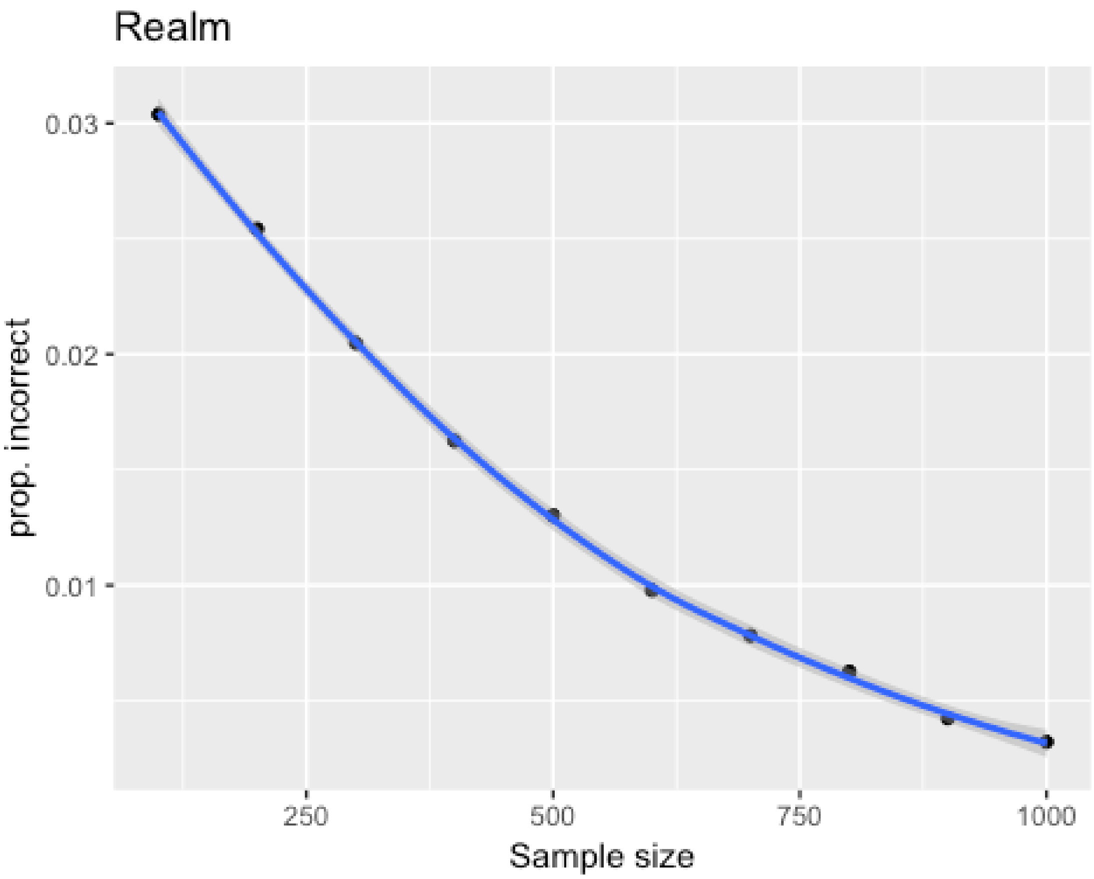
```
 # Compare these counts
 m_t_system = chisq.test(rbind(m_systems_count, m_systems_sample_count),
simulate.p.value = T)
 m_t_realm = chisq.test(rbind(m_realms_count, m_realms_sample_count),
simulate.p.value = T)
 m_t_order = chisq.test(rbind(m_order_count, m_sample_order_count),
simulate.p.value = T)

 # Record if the difference is significant
 m_p_system = m_t_system$p.value
 m_p_realm = m_t_realm$p.value
 m_p_order = m_t_order$p.value
 if (m_p_system <= thresh) m_k_system = m_k_system + 1
 if (m_p_realm <= thresh) m_k_realm = m_k_realm + 1
 if (m_p_order <= thresh) m_k_order = m_k_order + 1
 }
 # Store frequency of how many were significant.
 m_results_system[j] = m_k_system
 m_results_realm[j] = m_k_realm
 m_results_order[j] = m_k_order
}

m_results_system_df = data.frame(sample_size=samps, k =
m_results_system, N_REP=N_REP)
write.csv(m_results_system_df, file=paste("mammal_system.csv"))

m_results_realm_df = data.frame(sample_size=samps, k = m_results_realm,
N_REP=N_REP)
write.csv(m_results_realm_df, file=paste("mammal_realms.csv"))

m_results_order_df = data.frame(sample_size=samps, k = m_results_order,
N_REP=N_REP)
write.csv(m_results_order_df, file=paste("mammal_taxon.csv"))
```

We can then visualise the percentage of random samples with a p value bellow 0.05 for a set of sample sizes

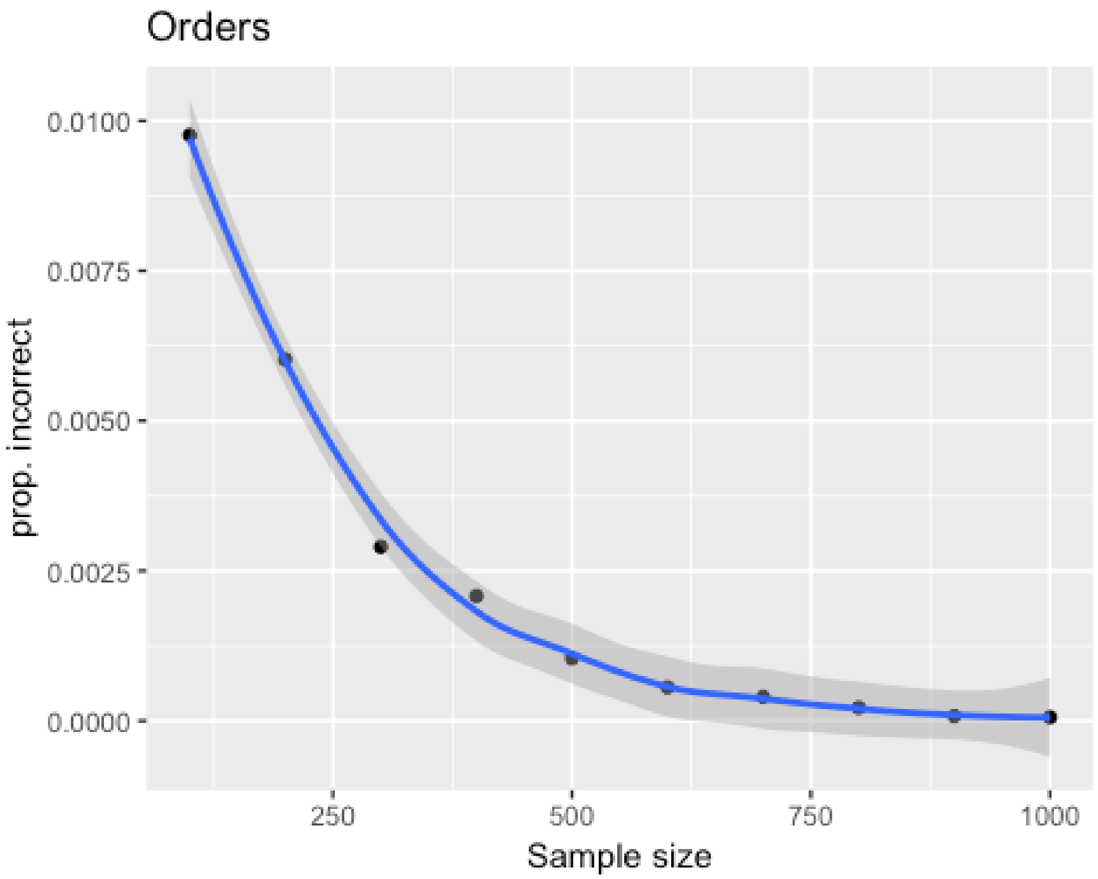
```
mammal_system <- read.csv("mammal_system.csv")
ggplot(mammal_system, aes(x=sample_size, y=(k/N_REP))) + geom_point() +
geom_smooth() + ggtitle("Systems") + xlab("Sample size") + ylab("prop.
incorrect")
```



```
mammal_realms <- read.csv("mammal_realms.csv")
ggplot(mammal_realms, aes(x=sample_size, y=(k/N_REP))) + geom_point() +
geom_smooth() + ggtitle("Realm") + xlab("Sample size") + ylab("prop.
incorrect")
```



```
mammal_taxon <- read.csv("mammal_taxon.csv")
ggplot(mammal_taxon, aes(x=sample_size, y=(k/N_REP))) + geom_point() +
geom_smooth() + ggtitle("Orders") + xlab("Sample size") + ylab("prop.
incorrect")
```



Amphibians

Using the same analysis on amphibian data

```
all_data <-read.csv("All_Red_List_data.csv", stringsAsFactors=FALSE)
amph_data = subset(all_data, Class=="AMPHIBIA")
write.csv(amph_data, "Amph_Red_List_data.csv")

#Data wrangling from all the IUCN data
amph <-read.csv("Amph_Red_List_data.csv", stringsAsFactors = FALSE)
amph_loc <- amph %>%
 select(Class, Order, Family, Species, Regions)
amph_loc <- unique(amph_loc)

#Convert the geographical information in the data set into the
biogeographical realms
amph_loc[amph_loc=="Europe"] <- c("Palearctic")
amph_loc[amph_loc=="North Africa"] <- c("Palearctic")
```

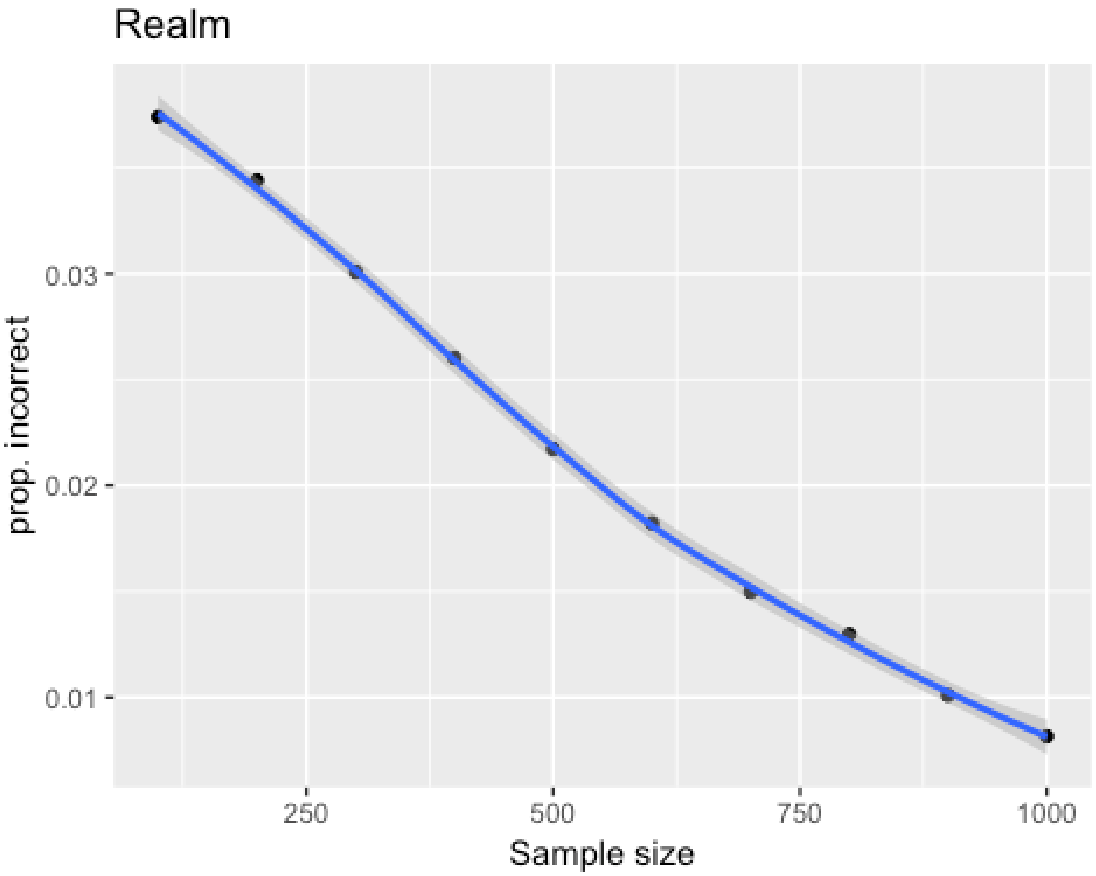
```
amph_loc[amph_loc=="North Asia"] <- c("Palearctic")
amph_loc[amph_loc=="West and Central Asia"] <- c("Palearctic")
amph_loc[amph_loc=="Sub-Saharan Africa"] <- c("Afrotropical")
amph_loc[amph_loc=="South and Southeast Asia"] <- c("Indomalayan")
amph_loc[amph_loc=="Oceania"] <- c("Australasia/Oceania")
amph_loc[amph_loc=="North America"] <- c("Neartic")
amph_loc[amph_loc=="South America"] <- c("Neotropical")
amph_loc[amph_loc=="Caribbean Islands"] <- c("Neotropical")
amph_loc[amph_loc=="Mesoamerica"] <- c("Neotropical")

#Environmental Systems were not tested as the vast majority of amphibean
live in fresh water environments.

#testing biogeographical realms
amph_reg <- table(amph_loc$Species, amph_loc$Regions)
amph_reg[amph_reg>1] <- 1
amph_realms = amph_reg[, c(2:8)]
amph_realms_count = colSums(amph_realms, na.rm = T)

##testing taxonomical orders
amph_ord <- table(amph_loc$Species, amph_loc$Order)
amph_ord[amph_ord>1] <- 1
amph_orders = amph_ord[, c(1:3)]
amph_order_count = colSums(amph_orders, na.rm = T)

# For 50000 replicates
N_REP = 50000

#Setting threshold, sample size and number of repetitions
thresh = 0.05
samps = c(seq(100, 1000, by=100))

amph_results_realm = rep(NA, length(samps))
amph_results_order = rep(NA, length(samps))
```

```
for (j in 1:length(samps)) {
 SAMP_SIZE = samps[j]
 cat(sprintf("Sample size %d\n", SAMP_SIZE))

 amph_k_realm = 0
 amph_k_order = 0

 for (i in 1:N_REP) {

 amph_sample_idx_reg = sample(1:nrow(amph_realms), size = SAMP_SIZE)
 reg_amph_sample_data = amph_realms[amph_sample_idx_reg, ]

 amph_sample_idx_ord = sample(1:nrow(amph_orders), size = SAMP_SIZE)
 ord_amph_sample_data = amph_orders[amph_sample_idx_ord, ]

 # Count how many of each realm
 amph_realms_sample_count = colSums(reg_amph_sample_data[, c(1:7)])

 # Count how many of each order
 amph_order_sample_count = colSums(ord_amph_sample_data[, c(1:3)])

 # Compare these counts
 amph_t_realm = chisq.test(rbind(amph_realms_count,
amph_realms_sample_count), simulate.p.value = T)
 amph_t_order = chisq.test(rbind(amph_order_count,
amph_order_sample_count), simulate.p.value = T)

 # Record if the difference is significant
 amph_p_realm = amph_t_realm$p.value
 amph_p_order = amph_t_order$p.value
 if (amph_p_realm <= thresh) amph_k_realm = amph_k_realm + 1
 if (amph_p_order <= thresh) amph_k_order = amph_k_order + 1
 }
 # Store frequency of how many were significant.
 amph_results_realm[j] = amph_k_realm
 amph_results_order[j] = amph_k_order
```

```
}

Amph_results_realm_df = data.frame(sample_size=samps, k =
amph_results_realm, N_REP=N_REP)
write.csv(Amph_results_realm_df, file=paste("amphibian_realms.csv"))
Amph_results_order_df = data.frame(sample_size=samps, k =
amph_results_order, N_REP=N_REP)
write.csv(Amph_results_order_df, file=paste("amphibian_taxon.csv"))
```

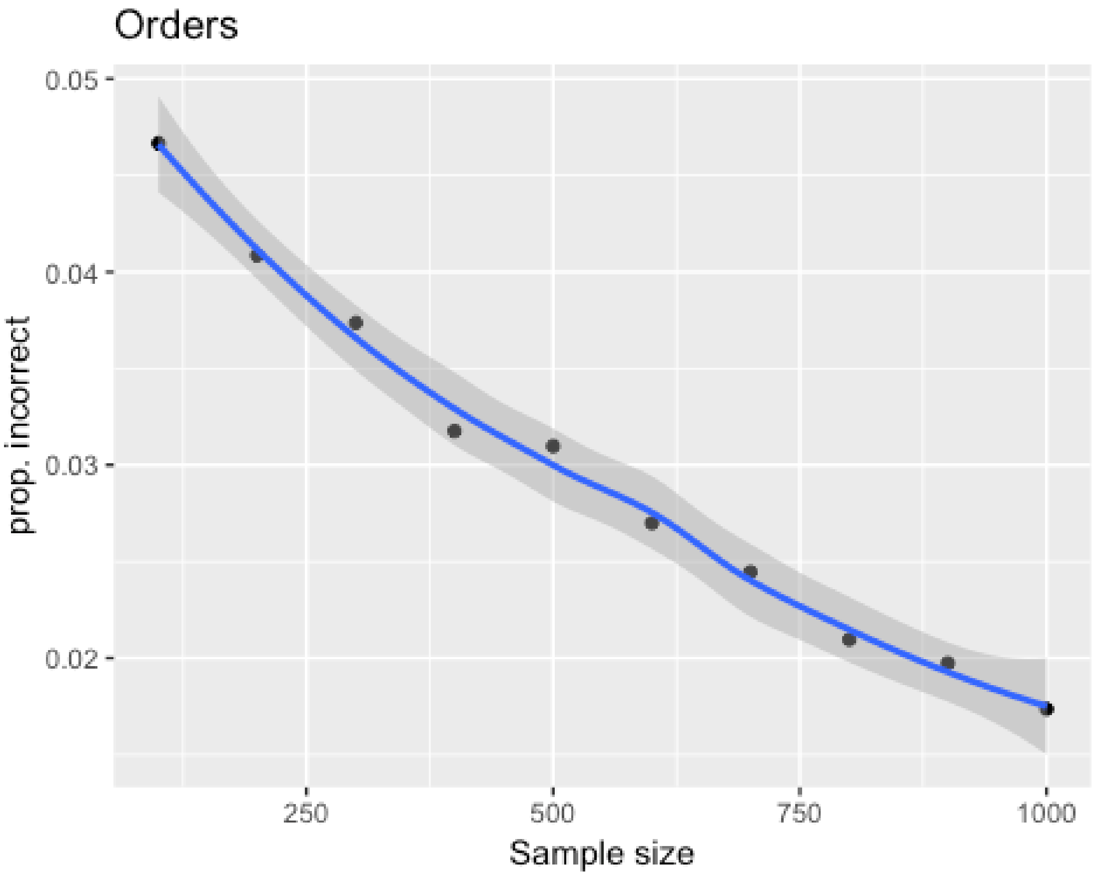
We can then visualise the percentage of random samples with a p value bellow 0.05 for a set of sample sizes

```
Amph_results_realm_df <- read.csv("amphibian_realms.csv")
ggplot(Amph_results_realm_df, aes(x=sample_size, y=(k/N_REP))) +
geom_point() + geom_smooth() + ggtitle("Realm") + xlab("Sample size") +
ylab("prop. incorrect")
```



```
Amph_results_order_df <- read.csv("amphibian_taxon.csv")
ggplot(Amph_results_order_df, aes(x=sample_size, y=(k/N_REP))) +
```

```
geom_point() + geom_smooth() + ggtitle("Orders") + xlab("Sample size") +
ylab("prop. incorrect")
```



Corals

Using the same analysis on corals data

```
all_data <-read.csv("All_Red_List_data.csv", stringsAsFactors=FALSE)
amph_data = subset(all_data, Class=="ANTHOZOA")
write.csv(amph_data, "Coral_Red_List_data.csv")

#Data wrangling from all the IUCN data
coral <-read.csv("Coral_Red_List_data.csv", as.is=T)
#Gather a coral dataset which contains data on taxonomy and
biogeographical realms
coral_loc <- coral %>%
 select(Class, Order, Family, Species, Regions)
unic_coral_loc <- unique(coral_loc)
#from this list several species where not included in the RLI
```

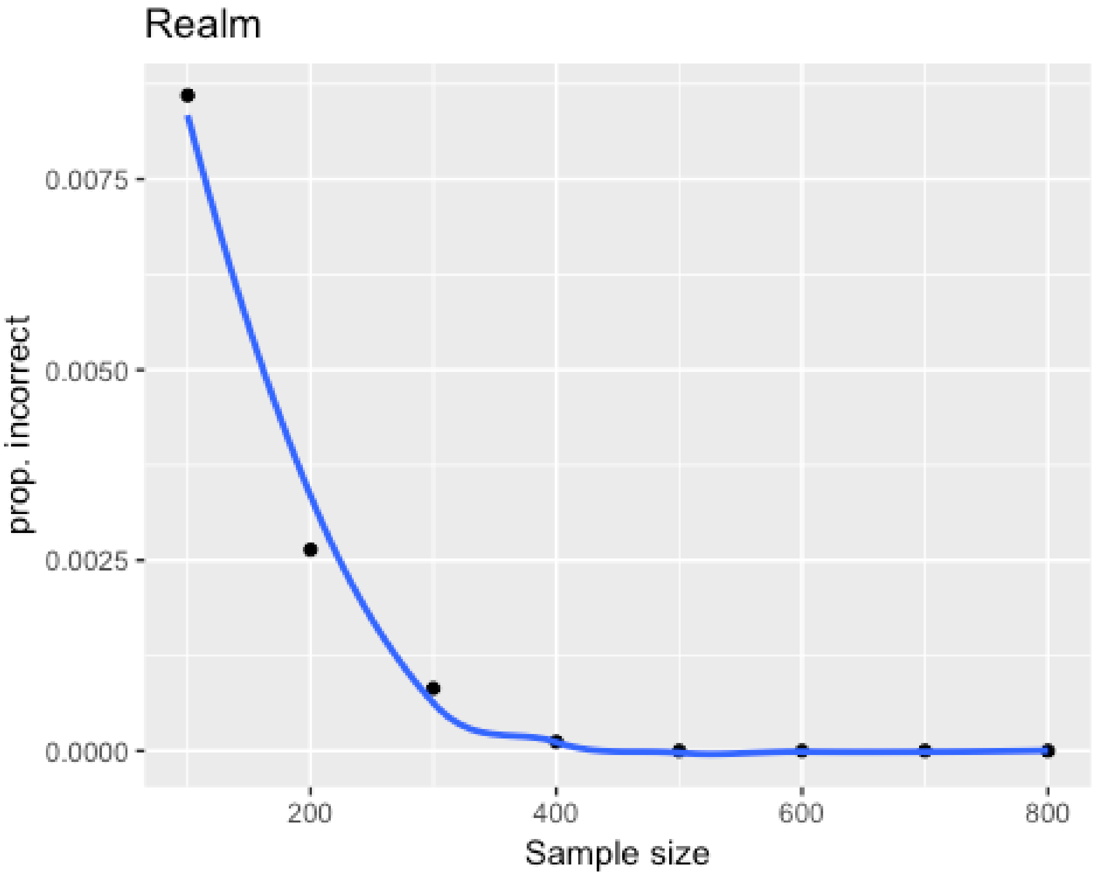
```
#therefore we intersected this list with the coral species in the RLI
Coral_sp <-read.csv("Coral_RLI.csv", as.is=T)
coral_list <- c(Coral_sp$Species)
coral_df <- intersect(unic_coral_loc$Species, Coral_sp$Species)
coral_df <- unic_coral_loc[unic_coral_loc$Species %in% coral_list,]

#testing variables

#Environmental Systems were not tested as all reef producing corals live in marine environments.

#testing biogeographical realms
c_realms <- table(coral_df$Species, coral_df$Regions)
c_realms[c_realms>1] <- 1
coral_realms = c_realms[, c(2:12)]
coral_realms_count = colSums(coral_realms, na.rm = T)



##testing taxonomical orders
coral_ord <- table(coral_df$Species, coral_df$Order)
coral_ord[coral_ord>1] <- 1
coral_ord = coral_ord[, c(1:3)]
coral_order_count = colSums(coral_ord, na.rm = T)
# coral_order_count shows that ALCYONACEA and HELIOPORACEA only have one sp. each, while the SCLERACTINIA order has 825 sp. we therefore used representativity family as a more meaningful higher taxonomic rank

##testing taxonomical families
c_fam <- table(coral_df$Species, coral_df$Family)
c_fam[c_fam>1] <- 1
coral_families = c_fam[, c(1:20)]
coral_families_count = colSums(coral_families, na.rm = T)

# For 50000 replicates
N_REP = 50000
```

```
#Setting threshold, sample size and number of repetitions
thresh = 0.05
#There are only 827 species in this groups, therefore simulations were tun up to 800
samps = c(seq(100, 800, by=100))

coral_results_realm = rep(NA, length(samps))
coral_results_fam = rep(NA, length(samps))

for (j in 1:length(samps)) {
 SAMP_SIZE = samps[j]
 cat(sprintf("Sample size %d\n", SAMP_SIZE))

 c_k_realm = 0
 c_k_fam = 0


 for (i in 1:N_REP) {

 coral_sample_idx_reg = sample(1:nrow(coral_realms), size = SAMP_SIZE)
 reg_coral_sample_data = coral_realms[coral_sample_idx_reg, ]


 coral_sample_idx_fam = sample(1:nrow(coral_families), size = SAMP_SIZE)
 fam_coral_sample_data = coral_families[coral_sample_idx_fam, ]



 # Count how many of each realm in the sample
 c_sample_realms = colSums(reg_coral_sample_data[, c(1:11)])
 # Count how many of each families in the sample
 c_sample_fam = colSums(fam_coral_sample_data[, c(1:20)])

 # Compare these counts
 c_t_realm = chisq.test(rbind(coral_realms_count, c_sample_realms),
```

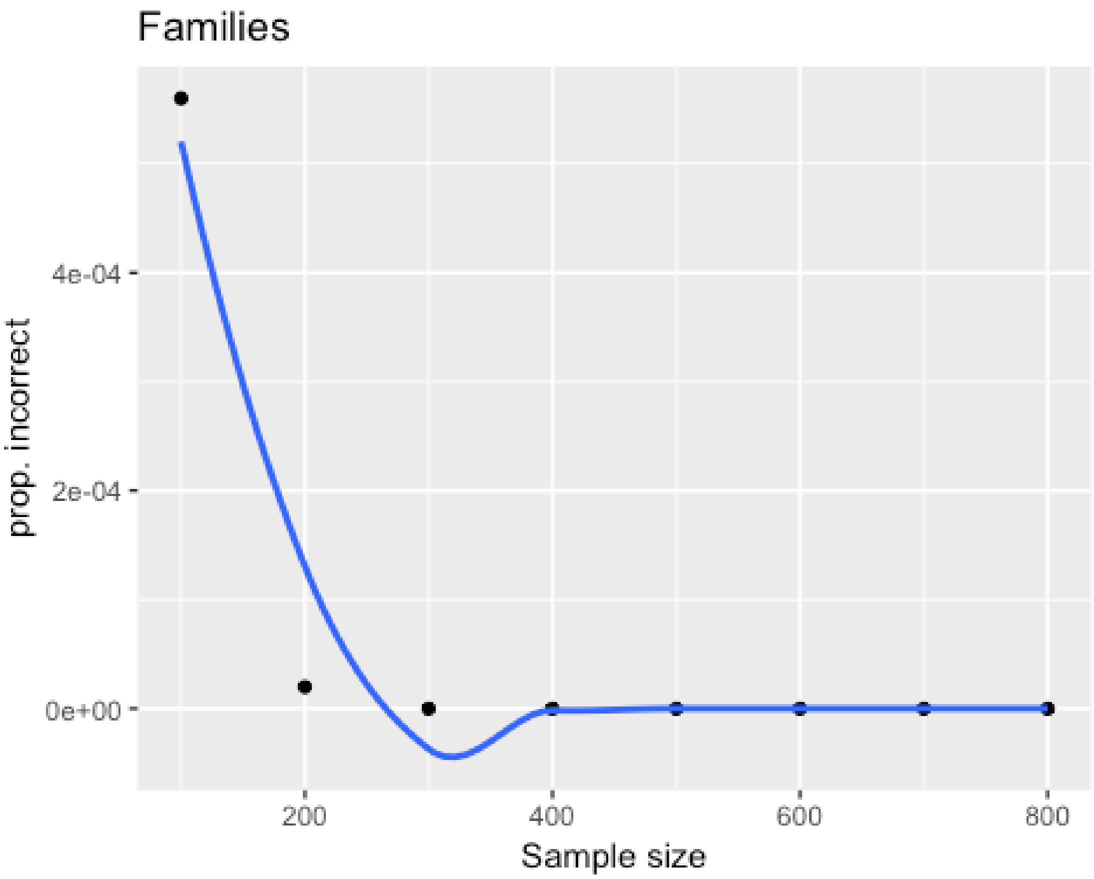
```
simulate.p.value = T)
 c_t_fam = chisq.test(rbind(coral_families_count, c_sample_fam),
simulate.p.value = T)

 # Record if the difference is significant

 c_p_realm = c_t_realm$p.value
 c_p_fam = c_t_fam$p.value
 if (c_p_realm <= thresh) c_k_realm = c_k_realm + 1
 if (c_p_fam <= thresh) c_k_fam = c_k_fam + 1
 }
 # Store frequency of how many were significant.
 coral_results_realm[j] = c_k_realm
 coral_results_fam[j] = c_k_fam
}

coral_results_realm_df = data.frame(sample_size=samps, k =
coral_results_realm, N_REP=N_REP)
write.csv(coral_results_realm_df, file=paste("coral_realms.csv"))
coral_results_families_df = data.frame(sample_size=samps, k =
coral_results_fam, N_REP=N_REP)
write.csv(coral_results_families_df, file=paste("coral_taxon.csv"))
```

We can then visualise the percentage of random samples with a p value bellow 0.05 for a set of sample sizes

```
coral_results_realm_df <- read.csv("coral_realms.csv")

ggplot(coral_results_realm_df, aes(x=sample_size, y=(k/N_REP))) +
geom_point() + geom_smooth(se=FALSE) + ggtitle("Realm") + xlab("Sample
size") + ylab("prop. incorrect")
```



```
coral_results_families_df <- read.csv("coral_taxon.csv")
ggplot(coral_results_families_df, aes(x=sample_size, y=(k/N_REP))) +
geom_point() + geom_smooth(se=FALSE) + ggtitle("Families") +
xlab("Sample size") + ylab("prop. incorrect")
```



Cycads

Using the same analysis on cycad data

```
all_data <-read.csv("All_Red_List_data.csv", stringsAsFactors=FALSE)
amph_data = subset(all_data, Class=="CYCADOPSIDA")
write.csv(amph_data, "Cycad_Red_List_data.csv")

#Data wrangling from all the IUCN data
Cycad <-read.csv("Cycad_Red_List_data.csv", as.is=T)
#Gather a coral dataset which contains data on taxonomy and
biogeographical realms
Cycad_loc <- Cycad %>%
 select(Class, Order, Family, Species, Regions)
unic_Cycad_loc <- unique(Cycad_loc)

#list of coral species in the SRLI
cycad_data <- read.csv("Cycad_RLI.csv", skip = 1, as.is=T)
```

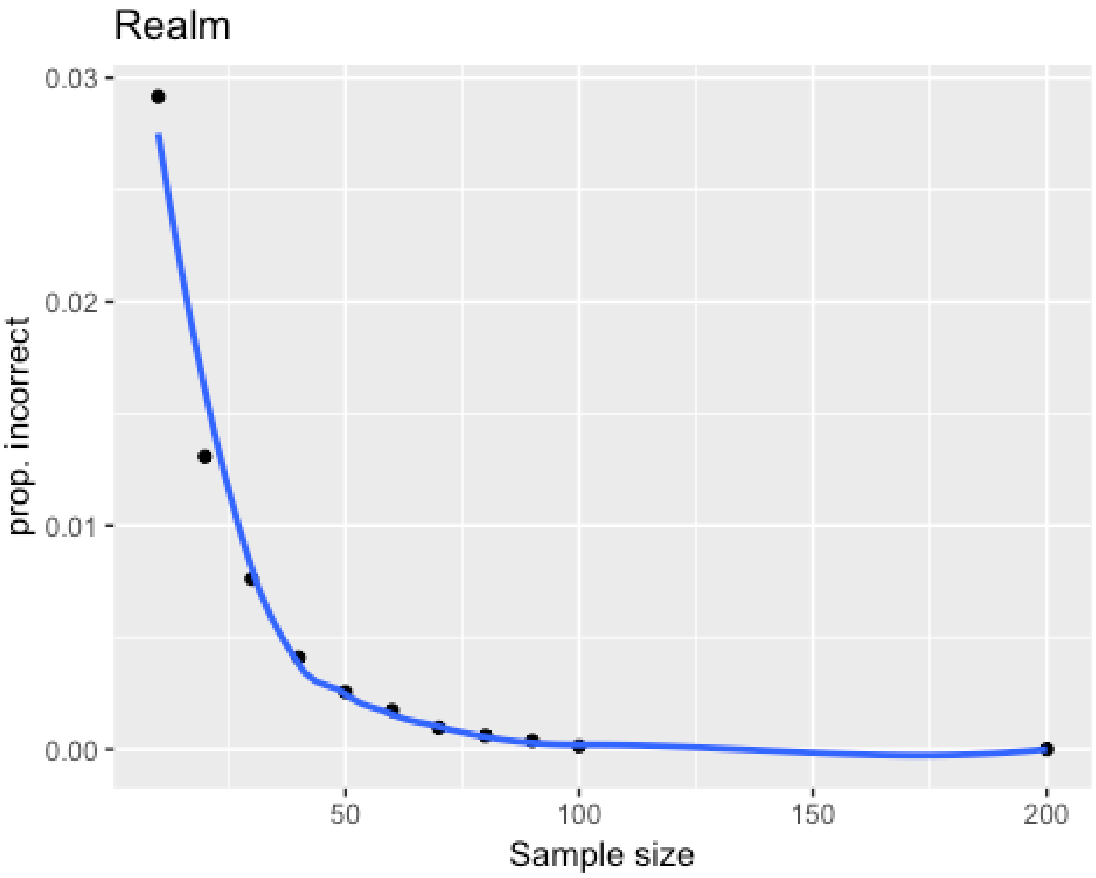
```r
#colnames(cycad_data) <- cycad_data[1,]
cycad_data <- cycad_data[2:dim(cycad_data)[1],]
cycads_list <- cycad_data$Scientific.name

cycad_df <- unic_Cycad_loc[unic_Cycad_loc$Species %in% cycads_list,]



#testing realms
cy_realms <- table(cycad_df$Species, cycad_df$Regions)
cy_realms[cy_realms>1] <- 1
cycad_realms = cy_realms[, c(1:8)]
cycad_realms_count = colSums(cycad_realms, na.rm = T)

#testing orders
cy_fam <- table(cycad_df$Species, cycad_df$Family)
cy_fam[cy_fam>1] <- 1
cycad_fam = cy_fam[, c(1:3)]
cycad_fam_count = colSums(cycad_fam, na.rm = T)

# For 50000 replicates
N_REP = 50000

#Setting threshold
thresh = 0.05

#There are only 325 species in this groups, therefore simulations were
tun up to 200
samps = c(seq(10, 90, by=10), seq(100, 200, by=100))
cycad_results_realms = rep(NA, length(samps))
cycad_results_fam = rep(NA, length(samps))




for (j in 1:length(samps)) {
 SAMP_SIZE = samps[j]
 cat(sprintf("Sample size %d\n", SAMP_SIZE))
```

```
 cy_k_realm = 0
 cy_k_fam = 0

 # For 1000 replicates
 for (i in 1:N_REP) {


 cycad_sample_realms_idx = sample(1:nrow(cycad_realms), size =
SAMP_SIZE)
 realms_cycads_sample_data = cycad_realms[cycad_sample_realms_idx, ]

 cycad_sample_fam_idx = sample(1:nrow(cycad_fam), size = SAMP_SIZE)
 fam_cycad_sample_data = cycad_fam[cycad_sample_fam_idx, ]

 # Count how many of each realm
 cy_sample_realms = colSums(realms_cycads_sample_data[, c(1:8)])

 # Count how mny of each order
 cy_sample_fam = colSums(fam_cycad_sample_data[, c(1:3)])

 # Compare these counts
 cy_t_realm = chisq.test(rbind(cycad_realms_count, cy_sample_realms),
simulate.p.value = T)
 cy_t_order = chisq.test(rbind(cycad_fam_count, cy_sample_fam),
simulate.p.value = T)

 # Record if the difference is significant

 cy_p_realm = cy_t_realm$p.value
 cy_p_order = cy_t_order$p.value
 if (cy_p_realm <= thresh) cy_k_realm = cy_k_realm + 1
 if (cy_p_order <= thresh) cy_k_fam = cy_k_fam + 1
 }
 # Store frequency of how many were significant.
 cycad_results_realms[j] = cy_k_realm
```

```
 cycad_results_fam[j] = cy_k_fam
}

cycad_results_realm_df = data.frame(sample_size=samps, k =
cycad_results_realms, N_REP=N_REP)
write.csv(cycad_results_realm_df, file=paste("cycad_realms.csv"))
cycad_results_fam_df = data.frame(sample_size=samps, k =
cycad_results_fam, N_REP=N_REP)
write.csv(cycad_results_fam_df, file=paste("cycad_taxon.csv"))
```

We can then visualise the percentage of random samples with a p value bellow 0.05 for a set of sample sizes

```
cycad_results_realm_df <- read.csv("cycad_realms.csv")
ggplot(cycad_results_realm_df, aes(x=sample_size, y=(k/N_REP))) +
geom_point() + geom_smooth(se=FALSE) + ggtitle("Realm") + xlab("Sample
size") + ylab("prop. incorrect")
```



```
cycad_results_fam_df <- read.csv("cycad_taxon.csv")
ggplot(cycad_results_fam_df, aes(x=sample_size, y=(k/N_REP))) +
geom_point() + geom_smooth(se=FALSE) + ggtitle("Families") +
xlab("Sample size") + ylab("prop. incorrect")
```

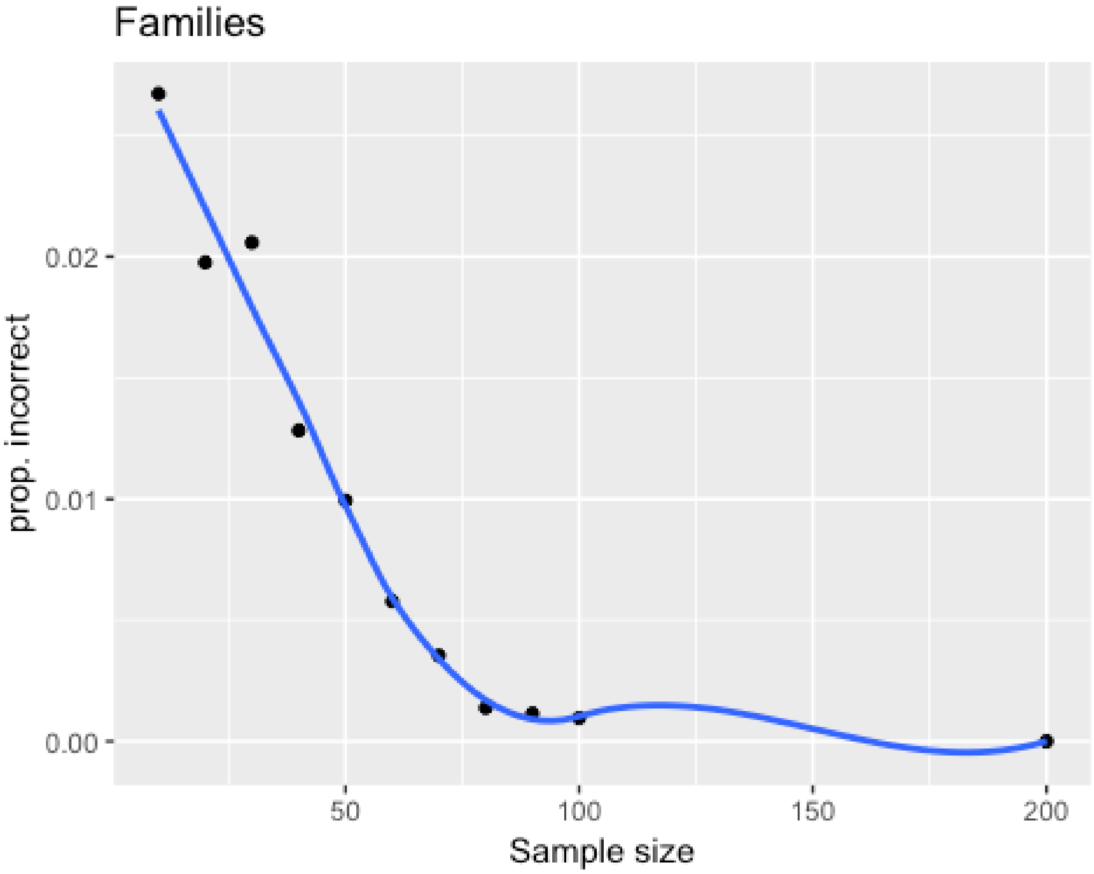


Figure S2.6

Combining the analysis for all these taxonomic groups and variables into a single graph produces Figure S2.6.

Chapter 3

Broadening the monitoring of global biodiversity: Using the sampled approach to measure the percentage of threatened species

Manuscript in preparation: Henriques, S., Böhm, M., Cornford, R. & Freeman, R.

Abstract

In the lead-up to the 15th Conference of the Parties to the Convention on Biological Diversity, where new biodiversity targets will be set to guide future conservation action, it is imperative that current indicators are critically reviewed to validate how well they have informed us in the past, where we stand now and how best to allocate resources going forward. One of the most promising metrics, with the potential to successfully address current knowledge gaps in extinction risk, is the sampled approach to the Red List Index. However, a decade after its inception, despite remarkable efforts towards producing a baseline for several taxonomic groups, no re-assessment of those samples has yet been produced to fully implement the protocol and detect extinction risk trends. Meanwhile, the single point generated to become a baseline has been used to compare the extinction risk between taxonomic groups and to estimate the proportion of threatened species globally, a metric that this indicator was never tested to address.

Using a wide data set of all comprehensively assessed taxonomic groups, we found that in random samples with as few as 200 non-DD species, the estimated proportion of threatened species was within +/- 5% of the known ‘true’ value for the whole group. However, when using a statistical approach to calculate the margin of error of incremental sample sizes in simulations, we found that 200 sp. can misestimate the threatened proportion of the whole group by 6% when the distribution of the threatened to non-threatened is 50/50. Because these errors represent a deviation from the true proportion of threatened species, this, in turn, reflects on the number of species we infer to be threatened, potentially impacting the current estimate of globally threatened species by at least a quarter of a million.

Sampled assessments are a remarkably versatile indicator, but the error of the estimate should be carefully considered when interpreting results and comparing different taxonomic groups, as there is a trade-off between how accurate we want to be and the number of assessments needed to reach that goal.

Keywords. Aichi Biodiversity targets, CBD 2020, extinction risk, IUCN Red List, post-2020 Global Biodiversity Framework, RLI, sRLI, Target 12

3.1. Introduction

Under the current biodiversity crisis, one of the most overwhelming statistics is that one million species are estimated to be at risk of extinction (IPBES 2019b). However, the number itself is too large to even contemplate because the consequences of such loss would be felt across society at a global scale (Flessa et al. 1986; Sodhi et al. 2009; Kuipers et al. 2019; Tripodi et al. 2019; Colléony et al. 2020; Mathiasson & Rehan 2020). Understanding where this value originated is paramount not only to halt what is placing all these species at risk but also to develop strategies to protect them in the future and reverse current trends (Mace et al. 2018; Leclère et al. 2020).

The most widely used method to assess the extinction risk of species, and the one used in the aforementioned estimate, is the IUCN Red List of Threatened Species (Purvis 2019), which applies quantitative criteria to place species in extinction risk categories (Mace et al. 2008; IUCN 2020). These categories range from Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), and Critically Endangered (CR), to Extinct in the Wild (EW) and Extinct (EX), ordered by increasing species extinction risk. Additionally, species can be classified as Data Deficient (DD) when available information is insufficient to assign a narrow range of risk, i.e., they are as likely to be LC, CR, or even EX (IUCN Standards and Petitions Subcommittee 2016).

These categories can be used as a monitoring tool in a relatively complex approach following the RLI protocol (Butchart et al. 2004; Henriques et al. 2020), or in order to highlight the two main levels of extinction risk, species can simply be separated in a binary fashion between those in threatened categories (VU, EN, CR) and those in non-threatened categories (LC, NT). The resulting analysis of threatened species within a given group, presented as a percentage/proportion or a fraction (“*one in five of world's plant species at risk of extinction*”), is a simple but powerful approach to illustrate the level of threat to biodiversity, one that has been broadly used to engage with the broader public and policymakers (Vidal 2013; Carrington 2016; RBG Kew 2016). It is also the foundation for the estimate that one million species are at risk of extinction (IPBES 2019b), which was globally reported in the media (Figure 3.1).

Figure 3.1 - Television platforms presenting the IPBES report and emphasising the 1 million species findings: a) The Daily Show with Trevor Noah (USA); b) China Central Television (China); c) TV Brasil (Brazil); d) Rajya Sabha Television (India).

Several animal and plant groups have had comprehensive assessments of all their species' extinction risk under the IUCN Red List, and we have a solid understanding of the percentage of species threatened within them at the time of their last assessment (IUCN 2020). These groups include birds (Butchart et al. 2004), mammals (Hoffmann et al. 2010; Hoffmann et al. 2011), amphibians (Hoffmann et al. 2010), reef-building corals (Carpenter et al. 2008), and cycads (United Nations 2015). For other major taxonomic groups, only selected subsets have been comprehensively assessed; for example, we know the percentage of threatened species of the *Conus* genus (class Gastropoda; Peters et al. 2013), freshwater crabs and crayfish in the Crustacea (Cumberlidge et al. 2009; Richman et al. 2015), gymnosperm and several groups of dicot plants, such as birches, cacti, magnolias, maples, southern beeches, and teas (IUCN 2020). For other groups, a sampled approach was followed. The percentage of threatened species was measured in a random sample of pteridophytes, bryophytes, monocots and Fabales (Brummitt et al. 2015), Ascomycota fungi (Minter 2011), dragonflies (Clausnitzer et al. 2009), fish (Baillie et al. 2010; Hoffmann et al. 2010), reptiles (Böhm et al. 2013) and freshwater molluscs (Böhm et al. 2020). For others still, species extinction risk was assessed at specific geographic scales, such as all six families of European bees (Nieto et al. 2017), all six families of European butterflies, i.e. suborder Rhopalocera (Van Swaay et al. 2010) and European saproxylic beetles, i.e. species from twenty families that depend on wood decay (Calix et al. 2018).

The reported percentage of threatened species (under a binary threatened vs. non-threatened analysis) of all non-insect groups is, on average, approximately 25% (IUCN 2020). However, insects represent three-quarters of all species of animals and plants (Mora et al. 2011). In currently assessed insect groups, the percentage of threatened species ranges from approximately 9% to 17.9%, based on 9% of European butterflies being threatened, 9.2% of European bees, 13.9% of a global sample of dragonflies, 16% of European dragonflies, 17.9% European saproxylic beetles (Clausnitzer et al. 2009; Van Swaay et al. 2010; Nieto et al. 2017; Calix et al. 2018). Current estimates were made while recognising that the true proportion is "*a key uncertainty, but available evidence supports a tentative estimate of 10 per cent (established but incomplete)"* (IPBES 2019b), which was considered a cautious estimate (IPBES 2019a) and became used to calculate that based on the best estimate of the global number of insect species (Mora et al. 2011), at least half a million insect species are threatened (IPBES 2019b, a; Purvis 2019). The assumption that 25% of non-insect animals and plants are threatened adds another half a million species to this estimate, resulting in the widely publicised number of at least 1 million species at risk of extinction (Figure 3.1).

Because a third of taxonomic groups that supported this IPBES analysis were obtained via the sampled approach (Clausnitzer et al. 2009; Baillie et al. 2010; Hoffmann et al. 2010; Minter 2011; Böhm et al. 2013; Brummitt et al. 2015; Böhm et al. 2020), this protocol emerges as one of the most impactful towards this estimate. However, while for the taxonomic groups sampled, a set of important baselines have been set, the sampled approach to the RLI has so far not yet been implemented in its entirety by assessing the extinction risk of the sampled species more than once to detect trend direction (Henriques et al. 2020).

While the conservation research community strives to re-assess species extinction risk towards detecting trends, the proportion of threatened species in available samples has already been used to compare extinction risk between different taxonomic groups (IUCN 2020) or to inform valuable policy documents on global estimates of species under extinction risk (IPBES 2019b). However, the accurate depiction of a species group percentage of threatened species is not what the sampled approach to the Red List Index was developed or indeed tested for (Baillie et al. 2008). It is, therefore, important to understand the sample sizes that are suitable for estimating the percentage of threatened species, both to validate current estimates based on these values and to optimise the information gathered from future sampled approaches.

3.2. Methods

3.2.1. Data collection

We compiled the IUCN Red List categories of taxonomic groups that have been assessed for the RLI. This encompassed birds, mammals, amphibians, cycads and corals. We analysed each taxonomic group independently, subsetting its species according to whether they were non-threatened species (those assessed as Least Concern or Near Threatened) or threatened species (those assessed as Vulnerable, Endangered, Critically Endangered) (IUCN Standards and Petitions Committee 2019). We excluded species classified as Data Deficient and Extinct and Extinct in the Wild. Still, we considered species assessed as CR (possibly extinct) and CR (possibly extinct in the wild) threatened.

3.2.2. Proportion of threatened species, data simulations and confidence level

We calculated the proportion of threatened and non-threatened species based on the most recent assessment of taxonomic groups within our dataset (see methods 3.2.1): of 10894 birds in 2016, 5494 mammals in 2008, 4415 amphibians in 2004, 704 reef-building corals in 2008, 333 cycads in 2014. We tested each taxonomic group independently by estimating the proportion of threatened species in random subsets of increasing sample size, from 100 to 1000 species, at increments of 100, from the group’s species list without replacement. As corals and cycads had less than 1000 species, the maximum sample size analysed was capped at 700 for corals and 300 for cycads. To determine the variability of these estimates, we repeated this process 50,000 times. We then calculated the 0.05 and 0.95 quantiles or 0.025 and 0.975 of each sample size (using the generic function “quantile” in R), which represent our 90% and 95% confidence levels, respectively.

It is relevant to mention that when generating thousands of simulated samples and calculating the proportion of threatened species for each of them, a bell-shaped distribution of proportions is produced for each sample size (Figure 3.2). However, several factors impact the spread of those values. A large sample size, for example, will always have a lower spread than a small sample if both are taken from the same taxonomic group because very low or very high proportions of threatened species (the tails of the distribution spread) are less likely to be produced when more species get included in a sample, shortening the spread.

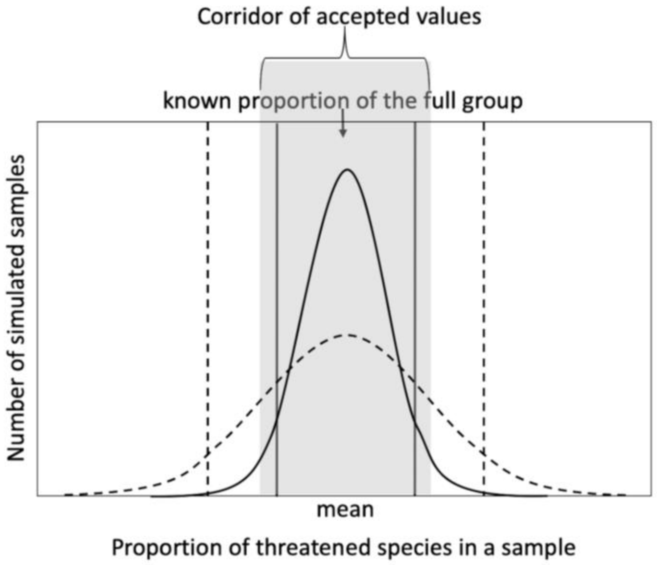


Figure 3.2 - Schematic distribution of the proportion of threatened species in two different sample sizes. The dashed line represents a sample with a widespread, and the solid line represents a sample with a low spread. Vertical lines represent the standard deviations that contain 95% of samples, where the sample size illustrated by a solid line meets our criterion (is within our corridor of accepted values), but the distribution indicated by the dashed line does not.

When we calculate the quantiles of our data to determine the 0.025 and 0.975 quantiles, we are, in effect, excluding the bell curve’s tail ends from our random 50,000 simulated samples. This example allows us to state that 95% of our simulated samples have a proportion of threatened species between value “ɑ” and proportion value “ϐ”. When a sample size of x species creates a distribution curve of proportions so narrow and tall that these ɑ and ϐ values are acceptably accurate representations of the known proportion of threatened species of the whole group (Figure 3.2), we can affirm that when assessing the extinction risk of x random species from an unstudied group with similar characteristics (i.e. statistical parameters), we can be 95% confident that the proportion of threatened species we record in our sample will be within an acceptable distance (margin or error) of the true proportion of the full group.

3.2.3. Margin of error and minimum sample size

Our question is, ‘How small can a sample be such that its confidence values lie close enough to our known true value?’. For ‘close enough’, margins of error were selected ± 2.5% or ± 5%, aiming to incorporate similar margins used in past analysis (Baillie et al. 2008; Henriques et al. 2020). These were used to build corridors around the known value within which results are accepted as sufficiently accurate, mirroring similar statistical corridor approaches (e.g. Schönbrodt & Perugini 2013). The upper threshold of the corridor was calculated by adding 0.025 to the known proportion of threatened species in that group, and we calculated the lower threshold by subtracting 0.025, which we will call the 2.5% corridor. We followed the same procedure, adding and subtracting 0.05 to the known proportion of threatened species to calculate the 5% corridor. This process aims to define the range of values that we would consider ‘close enough’ for our random sample.

Confidence intervals of our samples were estimated using the quantiles of our simulated samples (see section 3.2.2). We determined at which sample size the quantiles of the estimated proportion of threatened species lay within our 2.5% (or 5%) corridor. Similar to the current approach to establishing sample size to detect trend direction in the sampled Red List Index (Henriques et al. 2020), the smallest sample size to fall within each corridor was considered our minimum sample size.

3.2.4. Sample size calculator and statistical analysis

Statistically, a sample size which is representative of the proportion of a trait in an entire group (e.g. the proportion of threatened species) can be calculated as:

$$the\ sequals = \frac{z^2 \times\ p\ \times\ (1-p)}{me^2}$$

$$rss\ = \frac{ss}{1\ +\ \frac{(ss-1)}{population}}$$

where ss is a sample size parameter, which we use to calculate rss as the recommended sample size for a population of known size, as the number of species in the whole group; me is the margin of error which we set at either 0.05 or 0.025, as in our previous analysis (see section 3.2.3); p is the proportion of the target trait in the entire group as decimal, usually set at 0.5 once this value is rarely known beforehand, and 50/50 is the data distribution that requires the highest sample size to be confidently detected (see discussion for further details). Finally, z is the number of standard deviations a given proportion is away from the mean and was calculated as:

$$z = qnorm\ \left(0.5\ +\ \frac{confidence\ level}{200}\right)$$

where we used the standard “qnorm” function in R under two confidence levels, which we set at either 90% or 95%.

This approach allowed us to produce a baseline graph of incremental sample size, for p equal to 0.5, 0.25 or 0.1. Note that $p = 0.25$ will have the same distribution as $p = 0.75$, and $p = 0.1$ will have the same distribution as $p = 0.9$. Additionally we also plotted groups of increasing sample size, following the known proportion of threatened species in a number of selected taxonomic groups: cycads $p = 0.63$; amphibians $p = 0.42$; dicots (birches, cacti, magnolias, maples, southern beeches, and teas) $p = 0.36$; reptiles (chameleons, crocodiles and alligators, marine turtles, and sea snakes) $p = 0.34$; conifers $p = 0.34$, reef-forming corals $p = 0.33$; sharks, rays and chimeras $p = 0.30$; selected crustaceans (lobsters, freshwater crabs, freshwater crayfishes and freshwater shrimps) $p = 0.275$; mammals $p = 0.26$; birds $p = 0.14$; selected bony fishes (anchovies, angelfishes, billfishes, blennies, bonefishes, butterflyfishes, cornetfishes, groupers, parrotfishes, pufferfishes, sardines, sturgeons, surgeonfishes, tarpons, tunas, picarels, porgies, seahorses, seabreams, syngnathid fishes) $p = 0.08$; selected gastropods (cone snails) $p = 0.075$; and cephalopods (nautiluses, octopuses, squids) $p = 0.0015$.

3.2.5. Margin of error calculation

Mathematically, the sample size calculator presented in section 3.2.4 can become:

$$ss = \frac{sample \ - sample \ \times \ population}{sample - population}$$

$$me = \sqrt{\frac{z^2 \times \ p \ \times \ (1-p)}{ss}}$$

which we used to determine the margin of error me obtained for increasing $sample$ size from 100 to 1000 species at increments of 100, if the group's proportion of the trait being analysed was set as p equal 0.5, 0.25 or 0.1. As in the earlier example $p = 0.25 \ \Leftrightarrow \ p = \ 0.75$ and $p = 0.1 \ \Leftrightarrow \ p = \ 0.9$(see section 2.4). Finally, z was calculated under the confidence levels 90% and 95%, as in the previous analysis.

We used the resulting distribution of margins of error in increasing sample sizes to calculate how many species could potentially be misestimated as non-threatened for a case study of a megadiverse group with 50,000 species. We also followed a similar approach, under the set margin of errors of 0.5 and 0.025 (see section 3.2.3.), to determine for a range of taxonomic group sizes (from 1,000 to 350,000 species) how many species within those groups could potentially be misestimated as non-threatened.

3.3. Results

We found that with a 95% confidence level, random samples of 200 species of birds and cycads were within the 5% corridor, but for amphibians, corals and mammals, samples of 300 species were needed to meet the same threshold (Figure 3.3). When aiming to be within 2.5% of the known percentage of threatened species in a given group, with a 95% confidence level, we found that cycad samples of 300 species were within the 2.5% corridor, while for corals, birds, mammals and amphibians 500, 700, 800 and 900 species were needed, respectively (Figure 3.3).

We also found samples of as few as 200 species were within 5% of the known value for the whole group with 90% confidence, except for amphibians, where a sample size of 300 was solely able to meet the same thresholds (Figure 3.3). When aiming for the same confidence level to be within 2.5% of the known percentage of threatened species in a given group, we found that amphibian samples of 800 species were within this 2.5% corridor (Figure 3.3), whereas for mammals, birds, corals and cycads, 600, 500, 400 and 300 species were needed respectively (Figure 3.3).

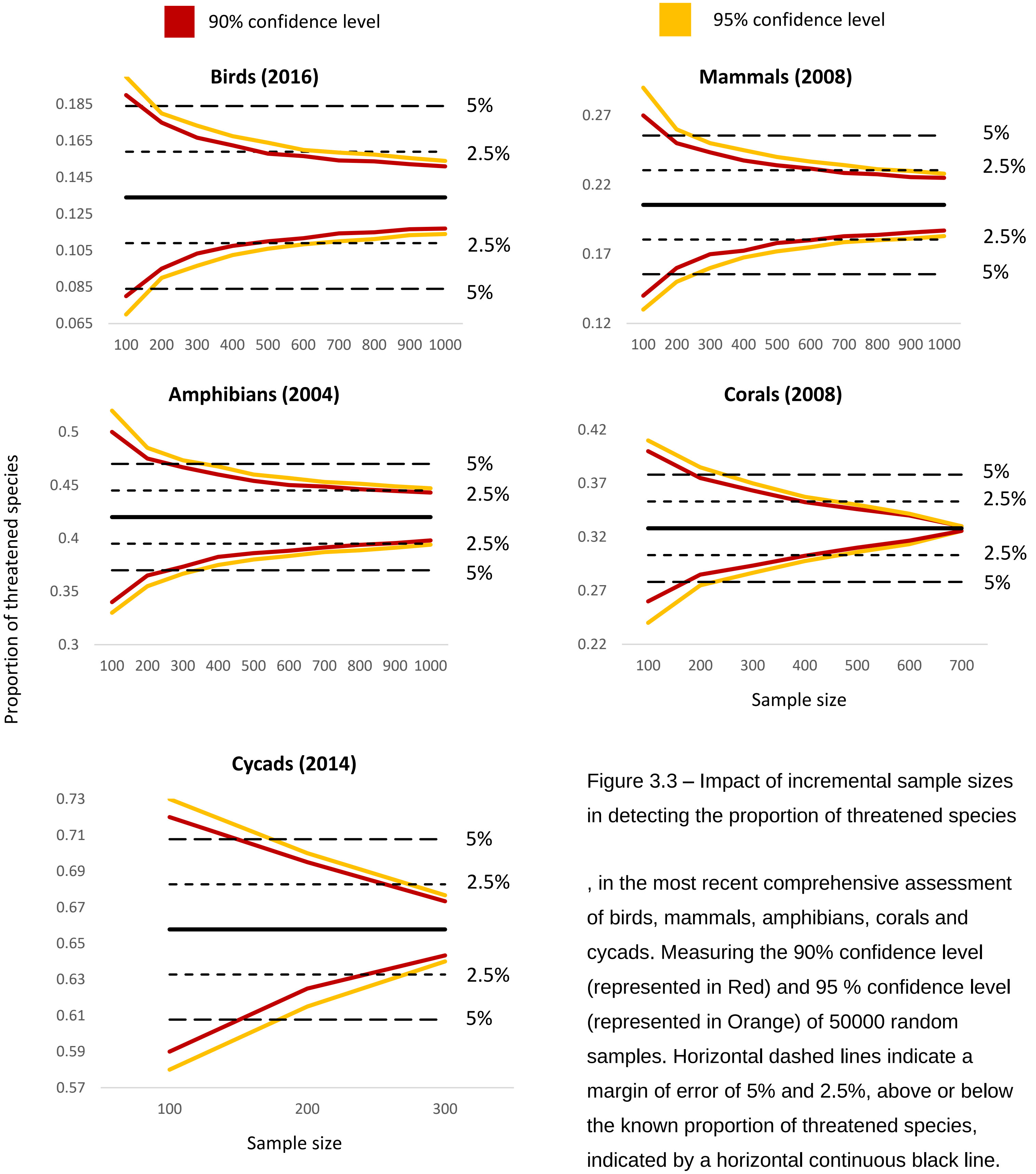


Figure 3.3 – Impact of incremental sample sizes in detecting the proportion of threatened species

, in the most recent comprehensive assessment of birds, mammals, amphibians, corals and cycads. Measuring the 90% confidence level (represented in Red) and 95 % confidence level (represented in Orange) of 50000 random samples. Horizontal dashed lines indicate a margin of error of 5% and 2.5%, above or below the known proportion of threatened species, indicated by a horizontal continuous black line.

When calculating the required sample size for a given group (see methods 3.2.4), we find that different assumptions about the underlying proportion of threatened species (i.e. 0.5, 0.25/0.75 or 0.1/0.9) greatly impact how large a sample has to be to detect a group's proportion of threatened species. For a 95% confidence level and a margin of error of 2.5%, a group with 10,000 species, for example, would need a sample of 1300 sp. under an underlying proportion of 0.5, a sample of 1000 sp. under 0.25/0.75, but only 500 sp. under 0.1/0.9 (Figure 3.4D). The group size does impact the sample size needed, but mostly for relatively low species numbers, as the curves start to reach an asymptote for groups with approximately 2,000 species for a 5% margin of error (Figure 3.4A, C) or approximately 6,000 species for a 2.5% margin of error (Figure 3.4B, D). Importantly, the impact of the margin of error is noticeable on sample size; once that meets 2.5% error, sample sizes asymptote at around 1000 sp. with 90% confidence (Figure 3.4B) or 1500 sp. with 95% confidence (Figure 3.4D), whereas to meet 5% error solely 300 sp. where needed, with 90% confidence (Figure 3.4A) or 400 sp, with 95% confidence (Figure 3.4B) For most taxonomic groups. Therefore, the margin of error is the key consideration for selecting a sample size.

When statistically determining the margin of error of incremental sample sizes, we saw a similar impact of the different underlying threatened species proportion, i.e. a 200 species sample with a 90% confidence level will have a 6% error under an underlying proportion of 0.5, an error of 5% under 0.25/0.75 and approximately 3% under 0.1/0.9 (Figure 3.5). In other words, the greater the proportion of threatened species within a group, the fewer species are needed to estimate that proportion. Sample size increments are also clearly impactful; a 200 species sample had a 0.06 error, whereas a 400 species sample had a 0.04 error (Figure 3.5A); although confidence level alone also impacts the margin of error, a 200 species sample had a 0.06 error at a 90% confidence level versus a 0.07 error at 95% confidence level (Figure 3.5A, B vs Figure 3.5C, D).

We also used this approach on the only insect group where the sRLI was implemented, dragonflies (Clausnitzer et al. 2009), where besides the baseline RLI value, this analysis also reported a 13.9% of threatened species, that could potentially be as low as 9% (if all DD species are not threatened) or as high as

44% (if all DD are threatened). When we add to these values the margin of error corresponding to a sample of 1500 sp., in a group with 5912 recognised sp., we find that the range of extinction risk in dragonflies increased the reported from 9% to 44%, to 6.6% to 46.5% with a 90% confidence, or 6.1% to 47% with a 95% confidence.

It is also important to understand what these margins of error mean in practice. We explored the number of species that may be misestimated as non-threatened when, in fact, they are threatened, or vice versa, for a megadiverse group of 50,000 species (Figure 3.5B, D). However, because the same error will represent different amounts of species for smaller or larger groups, it is important to note that in larger groups, like beetles (with approximately 243,000 described species but likely to hold 350.000 species), an error of 5% could represent 8,750 species being misestimated, whereas, in a group like dragonflies with 3,000 species, the same 5% error could represent 75 species (Table 3.1).

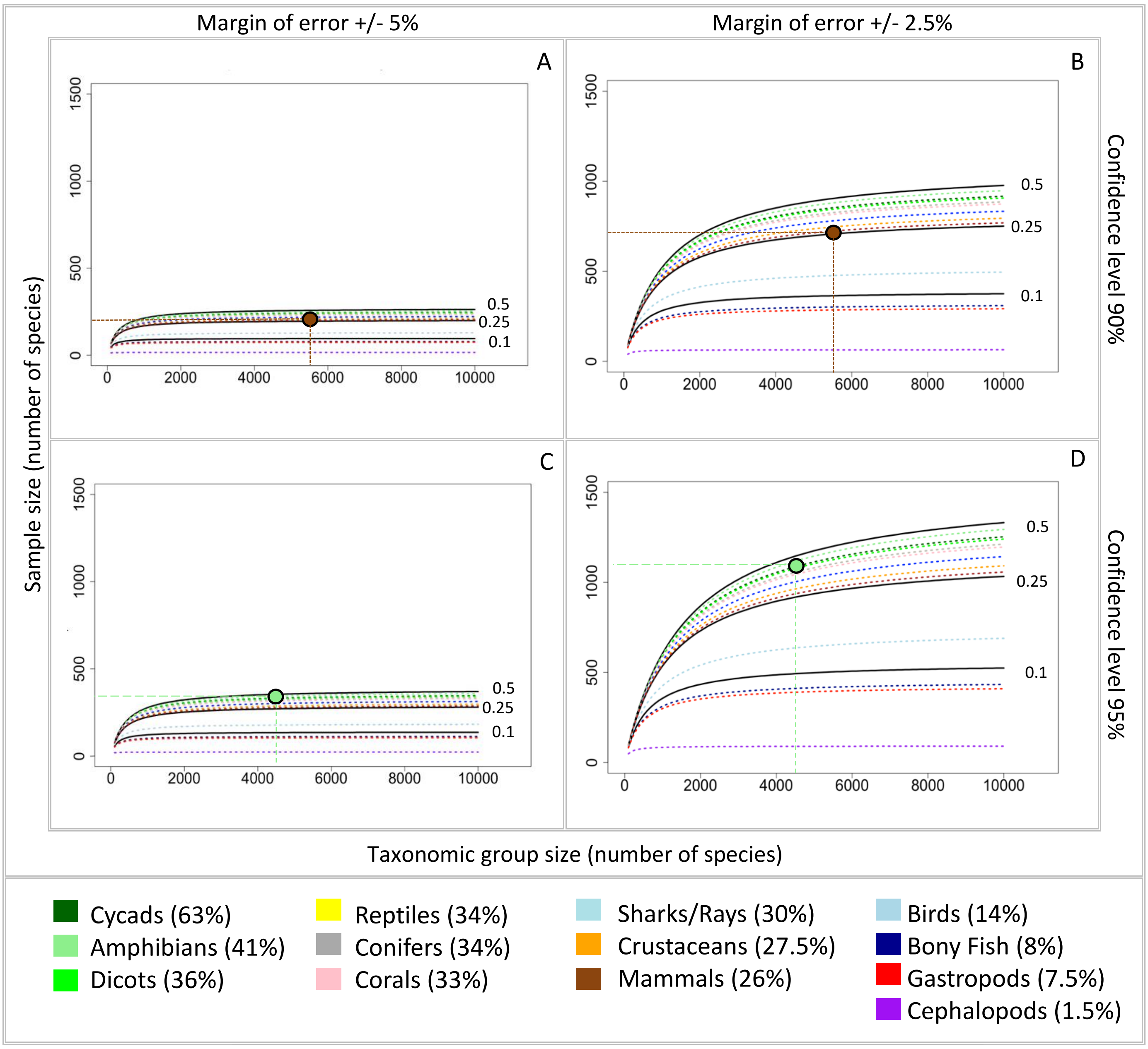


Figure 3.4 - Sample sizes (y-axis) needed to estimate the proportion of threatened species in taxonomic groups of different sizes (x-axis). With a margin of error of +/- 5% (A and C) or +/- 2.5% (B and D) and a confidence level of 90% (A and B) or 95% (C and D). Different underlying proportions of 0.5, 0.25/0.75 or 0.1/0.9 threatened species are represented by continuous lines in black, and the proportion of threatened species in known animal and plant groups are represented by dotted lines in colour, following the colour chart at the bottom which also shows the percentage of threatened species between parentheses. Amphibians and mammals' number of species and correspondent sample size are represented by a large coloured dot (green and brown, respectively).

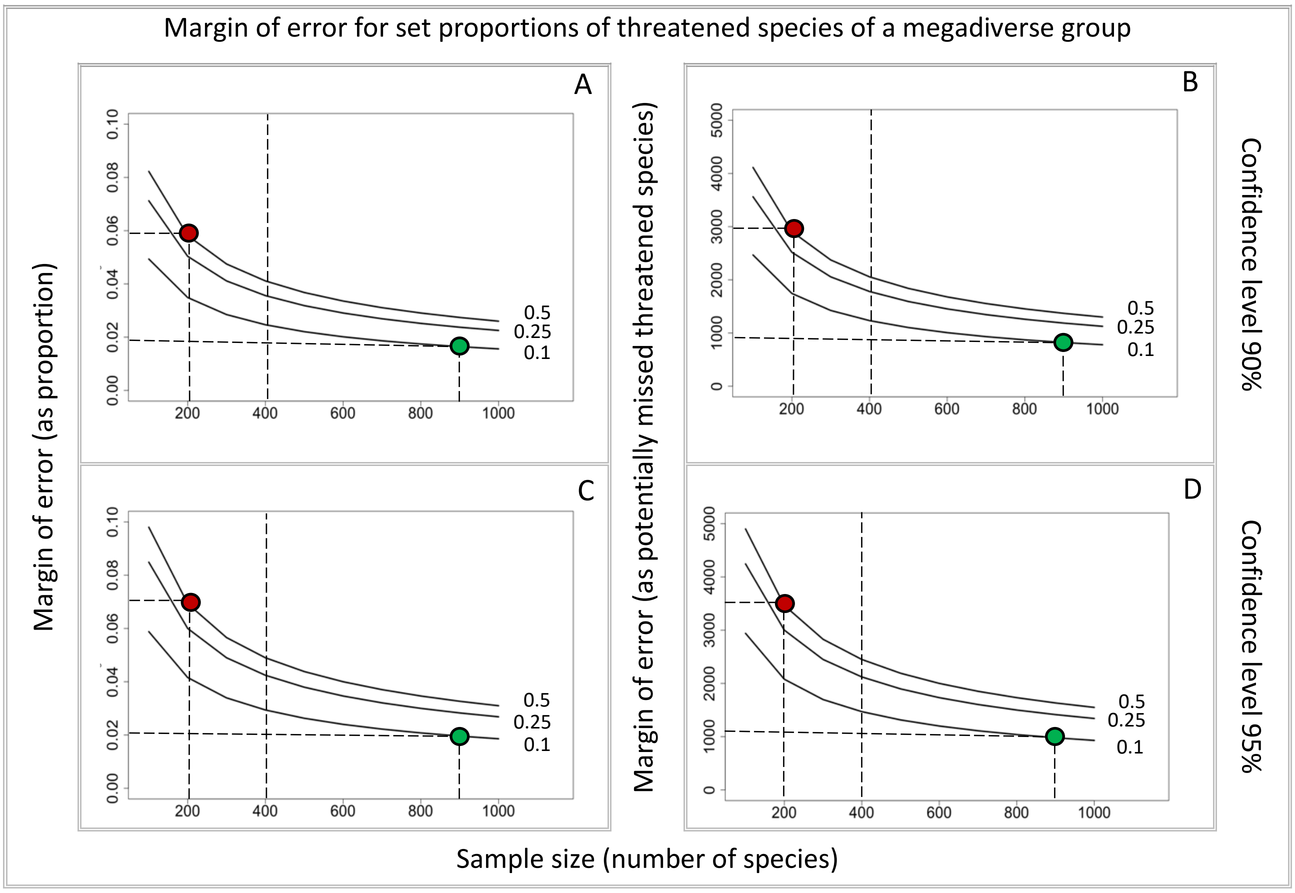


Figure 3.5 - Impact of incremental samples size (x-axis) under different response distributions of 0.5, 0.25/0.75 or 0.1/0.9 threatened species and a confidence level of 90% (A and B) or 95% (C and D). The margin of error (y-axis) was calculated as a proportion (A and C) or converted into the number of threatened species. That proportion entails we might misestimate in a megadiverse taxonomic group with 50000 species (B and D). Vertical dashed lines were set at recommended sample sizes of 200, 400, and 900 species from other relevant metrics in the literature (Henriques et al. 2020). Red indicates the highest error value for a small sample size, and green indicates the lower error value for a high sample size.

Table 3.1 - Number of threatened species that might be undetected in taxonomic groups with different species numbers (Taxonomic group) when considering a margin of error +/- 5% around the known proportion of threatened species or with a margin of error +/- 2.5%. Silhouettes represent groups that match those taxonomic group sizes: Odonata (dragonflies and allies) ca. 6,000 sp.; Agaricales (gilled mushroom) ca. 24,500 sp.; Asterales (sunflower and allies) ca. 39,000 sp.; Araneae (spiders) ca. 48,000 species; Lepidoptera (butterflies and allies), ca. 149,000 sp. and Coleoptera (beetles) ca. 243,000 (Roskov et al. 2020).

Taxonomic group	Potentially undetected threatened species	
	Margin of error +/-5%	Margin of error +/-2.5%
1000	25	5
10000	250	50
20000	500	100
30000	750	150
40000	1000	200
50000	1250	250
100000	2500	500
150000	3750	750
200000	5000	1000
250000	6250	1250
300000	7500	1500

3.4. Discussion

The percentage of threatened species is a powerful way to convey extinction risk and compare that risk between groups, one that can easily be grasped by anyone regardless of their level of expertise or overall knowledge of the biodiversity crisis at a time when conservation scientists are discussing the need for easy communicable target (Rounsevell et al. 2020).

Despite having been used as a communication tool to summarise complex environmental issues (Vidal 2013; Carrington 2016; RBG Kew 2016), functioning as a bridge between policy-making and science (IPBES 2019b), and having clear scalability as well as an ability to detect progress towards the policy goals the international community wants to achieve, the proportion or percentage of threatened species was never formally proposed as a biodiversity indicator (BIP 2020). This possibility may be worth considering in light of the often confusing array of indicators and the communications issues this can cause. The current sampled approach to the Red List Index (RLI) was originally tested to detect trends (Baillie et al. 2008; Henriques et al. 2020) and provides an existing and direct process to estimate the percentage of threatened species.

When testing what sample size can accurately estimate the known proportion of threatened species, we found that for all the taxonomic groups where we tested this approach (amphibians, birds, corals, cycads and mammals), 900 non-DD species were sufficient to detect the proportion of threatened species within a 2.5% corridor with a 95% confidence level. This aligns with the original sampled red-list protocol and suggests that many of the existing sampled assessments are within 2.5% of their true value (with 95% confidence).

Using a less strict accuracy criterion - aiming for 90% of our random samples to be within a 5% corridor, only 200 species samples were sufficient for most target groups. In contrast, solely amphibians (the group with a proportion of threatened species nearest to 50%) need 300 species (Figure 3.3). A 95% confidence interval was selected because it has been widely used in the literature, including to inform sample size recommendations for the sRLI (Baillie et al. 2008), whereas we used a 90% confidence level to show how it impacted sample size.

It was not surprising to find that under a 90% confidence level, we will always need smaller samples to meet the same threshold than under 95% (Figure 3.2) and that a narrower threshold around the true value (e.g. 2.5% corridor) will always need a higher sample to be met than a wider threshold (e.g. 5% corridor). This is one of the main trade-offs we identified in our analysis: deciding between how accurate we aim to be and how achievable it is to assess the sample size needed to reach that goal.

We estimated the impact of a range of recorded percentages of threatened species (full list of taxonomic groups in section 3.2.4) on the required sample size. We also simulated how sample size would be impacted if those same taxonomic groups were much larger (Figure 3.3). Our results show that the error we are willing to accept had the greatest impact on sample size but that when accepting a 90% probability of being within a 5% error of the true value, a 300 species sample would suffice, regardless of the size of the group or the proportion of threatened species within it (Figure 3.3A).

These results support the premise that samples as small as 200 species, which have shown to be informative of the overall group trend direction in available non-avian groups (Henriques et al. 2020), can also be informative of the group's percentage of threatened species within set margins of error and confidence levels (Figure 3.3A). The trade-off is that we can misestimate how many species are not threatened when, in fact, they could be.

When determining the margin of error for incremental sample sizes (see methods 2.5), in a hypothetical group that has 0.5 threatened species, we can be 95% confident that 200 species will produce an error as high as 0.07 (Figure 3.4C), or we can be 90% confident that the same sample size (200 sp.) can produce an error of 0.06 (Figure 3.4A). Similar impacts on error were also observed across a range of suggested sample sizes, such as those previously mentioned in the sampled approach to the RLI, i.e. 400 sp. and 900 sp. (Henriques et al. 2020). However, since the percentage of threatened species is often what we are yet to find out (it is unknown in unassessed groups), it further emphasises the premise that the main trade-off is between how large a sample size we can feasibly assess extinction risk for, and the error it might encompass. If converting into species numbers, in our proposed example of 50,000 species, we could potentially underestimate threatened species by 3500 sp. when solely analysing a 200 species sample (Figure 3.4D). Although it is simple to understand how a 0.07 error will represent a higher number of species in a large group of 50000 species (i.e. 3500) than in a smaller one of 1000 species (i.e. 70), this is an important point to raise, because these are the values that feed into impactful estimates like 1 million species are at risk of extinction (IPBES 2019b, a; Purvis 2019; Purvis et al. 2019).

Because half of the species driving this estimate are insects (IPBES 2019a), we found it particularly insightful to apply our findings to the case study of dragonflies, as it was the sole insect group that has calculated a baseline RLI based on a random global sample (Clausnitzer et al. 2009). When determining the margin of error in this sample, for p=0.5, we obtain a 0.029 margin of error, with a 95% confidence level, or 0.024 with a 90% confidence level. Under a best-case scenario, where all DD dragonflies in the global sample are not threatened, the proportion of threatened species could be as low as 9%, whereas in a similarly realistic scenario where all DD are threatened, it could be as high as 44% (Clausnitzer et al. 2009). This is important because the margin of error should not be added to the reported 13.9% of threatened species in this group but to the values the group could realistically have if the extinction risk of DD species was known, increasing the range from 9% to 44%, to a 6.6% to 46.5% with a 90% confidence, or 6.1% to 47% with a 95% confidence (Figure 3.6). We also calculated the margin of error for a global sample of reptiles and freshwater molluscs under 95% confidence, where we observed a similar impact on current estimates (Figure 3.6).

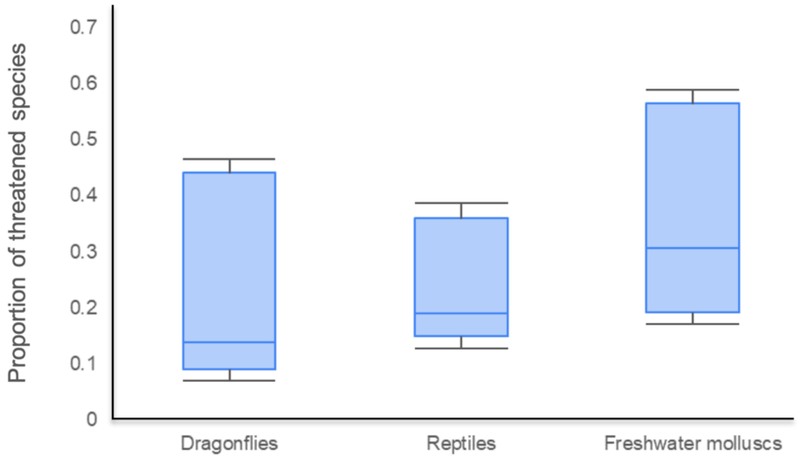


Figure 3.6 - Proportion of threatened species in random global samples of Dragonflies (Clausnitzer et al. 2009), Reptiles (Böhm et al. 2013) and Freshwater molluscs (Böhm et al. 2020). A horizontal blue line indicates a complete case analysis (proportion of threatened species in non-DD), the lower and upper edges of the blue rectangle indicate the results of best-worst sensitivity analyses (upper = all DD threatened, lower = all DD non-threatened), the margin of error bars indicated by black horizontal lines.

This has two important implications; firstly, it highlights that the impact of DD on the proportion of threatened species can be much greater than the statistically calculated margin of error. Secondly, it shows that margins of errors are not neglectable when applied to the proportion of threatened species, in the case of dragonflies above, in the example of a hypothetical megadiverse study group (Figure 3.5) or in the conservative global estimate that 10% of all global insects species are threatened (IPBES 2019b). In this last estimate, even if we assessed sizeable samples of 400 species, for example, within all insect orders, the relatively low margins of error of 0.05 such endeavour could generate (Figure 3.3C) could feasibly add a quarter of a million threatened species to the current estimate.

Data deficiency remains a significant cause of variation in our understanding of how many species are at risk of extinction. In the sampled assessment of dragonflies, for example, 35% of species were data deficient (Clausnitzer et al. 2009), and this may be the case in future sampled assessments for other groups. Our lack of knowledge is the biggest impediment to improving our estimates.

When sufficient species have been assessed, we found that how confident we need to be about our result matters, but that sample size is impacted more by how accurate we wish to be (our margin of error) than by our confidence intervals, as these quickly asymptote for larger groups. We hope that more taxonomic groups become assessed because, as we showed here, even small sample sizes (n=200) that can be used to detect a group's RLI trend in available non-avian groups (Henriques et al. 2020) can also be used to detect the group's proportion of threatened species. Our findings suggest that such a sample would have a margin of error of, at worst, 7% (using a 95% confidence interval). This may be sufficient for many groups, particularly as this error is likely to be outweighed by data deficiency.

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Chapter 4

Towards the conservation assessment of a megadiverse invertebrate group (Araneae, Arachnida)

Manuscript in preparation: Henriques, S., Böhm, M., Cardoso P. & Freeman, R.

Abstract

Spiders benefit human lives and play a crucial role in terrestrial ecosystems, so much so that life on Earth as we know it would not exist without them. Yet, they are often ignored or even actively persecuted. With growing evidence of global insect declines, spider populations are now expected to be steeply declining as well, although no attempt has been made to monitor their global extinction risk.

Here, we analyse for the first time the extinction risk of spiders from a randomly selected global species set (N=200). We applied the sampled approach to the Red List Index (sRLI) to produce a baseline from which to measure their conservation trend with future reassessments, and we compare this baseline to the status of other taxonomic groups. We found that 70.5% of our randomly selected species were so poorly known that they could not be assessed under IUCN Red List criteria and had to be classified as Data Deficient (DD), one of the highest percentages ever recorded for any group undertaking this analysis. Of the species that could be assessed, 93% were Least Concern (LC), and only four species could be confidently classified as threatened.

We discuss some of the most probable causes for the predominance of LC in non-DD assessments, the challenges of high DD percentage and the options to address it. We also analyse how current knowledge gaps impact sRLI implementation and list the obstacles that would most benefit from support when implementing this protocol in understudied groups.

Keywords: Aichi Biodiversity targets, extinction risk, IUCN Red List, Target 12, post-2020 Global Biodiversity Framework, sRLI, RLI, Red List Index

4.1 Introduction

Few living creatures are as underappreciated as spiders (Wiederhold & Bouchard 2014). Often feared and purposely killed, many do not realise that they protect our homes and crops by eating the animals that damage our property and contaminate our food (Bertone et al. 2016), having been recognised as crop biocontrol agents for decades (Riechert & Lockley 1984; Michalko et al. 2019). Despite the negative misperceptions that spiders conjure in the collective imagination, they have remarkable potential for biomaterial development (e.g. Shehata et al. 2019), they save human lives by eating deadly disease vectors (e.g. Nelson & Jackson 2006), and replicating their venom can save countless more, either as a pesticide (e.g. Lovett et al. 2019) or by developing new medical treatments (e.g. Chassagnon et al. 2017).

Spiders are also keystone species in natural ecosystems, as they consume at least 400 million tons of prey annually (Nyffeler & Birkhofer 2017), are a vital part of the diet of a wide range of animals themselves (Wise 1995; Foelix 2011), impact plant diversity by maintaining pollinator fitness (Schmitz & Suttle 2001; Morse 2007) and many bird species, including all hummingbirds, depend on their silk as nesting material (Hansell 2000). Even in cold environments like the arctic tundra, where spider diversity is often lower, and their size is far smaller than that of the relatively large tropical tarantulas, wolf spiders have 80 times more biomass than wolves in the same area (Koltz et al. 2018).

However, despite the crucial role of spiders in terrestrial ecosystems and their positive impact on human lives, arachnology as a research field has often been neglected and spiders remain fundamentally misunderstood. A number of relevant shortfalls have been identified for invertebrate conservation overall, such as lack of taxonomic expertise, lack of public awareness, low detectability, etc. (Cardoso et al. 2011; Perez et al. 2020) all of which sadly apply to most spider species. Adding irrational fears to these shortfalls is likely to further hinder arachnological studies (Vetter 2013); it is not surprising that spiders have been largely ignored from the extinction crisis debate and have neither been considered charismatic enough to be campaigned for nor have they been often included in invertebrate conservation, which has been primarily focused on target insect declines (Harvey et al. 2020).

Spider experts have been asked online what threats they consider more pressing (Branco & Cardoso 2020), and important insights have been gathered on particular regions measuring impacts of particular threats, such as trade (Fukushima et al. 2019), agricultural pesticides (Graf et al. 2019) or invasive species (Borges et al. 2020). However, an unbiased analysis of which threats are having a greater impact on the group globally, in which regions of the world or habitats they are faring better or worse, and how they may compare to other groups in which extinction risk has been analysed was never made.

While we wait for these questions to be addressed, spiders are likely undergoing a silent extinction because 1) food webs and trophic cascades make it inevitable that insect declines impact spider populations (Wise 1995); 2) human land use change, such as the conversion of natural habitat in urban or agricultural land, is more likely to impact small ectothermic predators like spiders (Newbold et al. 2020); and 3) in the only region where spider recording schemes have taken place, overall decline of invertebrates has in the recent past been driven mainly by spiders (Outhwaite et al. 2020).

Similar lines of evidence have already led to estimates of widespread extinction in other arachnid groups, such as mites (Sullivan & Ozman-Sullivan 2020), which, despite highlighting crucial knowledge gaps and shortfalls, is a limited approach towards measuring progress towards conservation success, as it does not list specific species that could be used as indicators of broader trends. The IUCN Red List of Threatened Species (hereafter Red List) could function as such an indicator.

The Red List applies quantitative criteria to place species in one of seven ranked extinction risk categories (Mace et al. 2008; IUCN 2012): Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Extinct in the Wild (EW) and Extinct (EX). These categories can be assigned incremental weights that can be averaged into an index to provide a single value summarising an entire group’s extinction risk, the Red List Index

(RLI). The RLI allows for an intuitive comparison of conservation status between groups and detects trends when measured multiple times (Butchart et al. 2004; Butchart et al. 2007; Butchart et al. 2010).

Although previous analyses have identified spiders as a priority group for IUCN Red Listing (Gerlach et al. 2014), implementing the RLI by assessing the Red List status of approximately fifty thousand recognised species of spiders (World Spider Catalog 2020) is not a feasible endeavour with the conservation resources currently available. However, a sampled approach to the RLI (sRLI) has the potential to overcome this obstacle by analysing a smaller sample of randomly selected species that represents the entire group (Henriques et al. 2020).

Several ongoing projects have used the sRLI to calculate the baseline values of several plant groups, namely pteridophytes, bryophytes, monocots and legumes (Brummitt et al. 2015), Ascomycota fungi (Minter 2011), and animal groups such as dragonflies (Clausnitzer et al. 2009), fish (Baillie et al. 2010; Hoffmann et al. 2010), reptiles (Böhm et al. 2013) and freshwater molluscs (Böhm et al. 2020). Work is in progress to implement it for butterflies (Lewis & Senior 2011), dung beetles (Collen et al. 2012) and grasshoppers (Hochkirch 2019 pers. comm.). Here, we implement the revised sRLI protocol (Henriques et al. 2020) for the first time, based on the extinction risk assessments of 200 randomly selected species of spiders (Seppälä et al. 2018a, b, c, d). This sample size, which if composed of only non-DD species, was observed to be sufficient to detect conservation trend direction in all currently available non-avian groups (Henriques et al. 2020).

We quantify the percentage of DD species in this understudied megadiverse group in order to estimate the required sample size to meet the minimum non-DD sample size, which was found to be informative in other taxa (Henriques et al. 2020). We also analyse the main causes of DD assessments and critically revise the main data gaps in order to best inform future resource allocation to improve the performance of an sRLI for spiders or other megadiverse poorly known groups.

4.2 Methods

4.2.1 Data collection

4.2.1.1. Species selection

A sample of 200 species of spiders was randomly selected from an up-to-date global database containing all spider species names recognised as valid at the time (Seppälä et al. 2018a, b, c, d; World Spider Catalog 2020). This sample size was recently shown to detect RLI trend direction correctly in all currently available non-avian groups with 95% accuracy (Henriques et al. 2020). Species considered valid during sample selection (2015) but which had been synonymised at the time of assessment were updated and replaced by the valid species name (see chapter 5 for more details), and species which became invalid during this time frame (e.g. *nomen dubium*) were removed from the sample and replaced by another randomly selected species following a refreshment approach (Mazen & Tong 2020; Taylor et al. 2020).

4.2.1.2. Species assessors and data sources

A group of ten arachnologists were given IUCN Red List assessment training during an IUCN Red List workshop. Ten spider species of our random subset of 200 species were assigned to each of these assessors, when possible, according to taxonomic or geographic expertise, leading to a total of 100 assessed spider species. The remaining 100 species were assessed by myself (SH) or by the first author of these species conservation profiles (Seppälä et al. 2018a, b, c, d). All data was revised by myself (SH), and the category was assigned by a single leading team member (PC) to ensure uniformity between the implementation of Criterion B. Species information such as habitat, behaviour, ecology and occurrence records were gathered from extensive literature review, which was based on the comprehensive list of taxonomic publications made available by the World Spider Catalogue at the time (World Spider Catalog 2016), as well as several other online bibliographical sources, including Google Scholar (scholar.google.co.uk), Biodiversity Heritage Library (www.biodiversitylibrary.org) and Web of Knowledge (www.webofknowledge.com). Several georeferenced records were also gathered from online datasets based on the compiled records made available at the Global Biodiversity Information Facility (www.gbif.org), as well as a number of other online sources, such as the British Spider and Harvestman Recording Scheme (www.srs.britishspiders.org.uk); the biological collections of the United States portal (www.symbiota.org); the interactive distribution atlas of Swiss animal species (www.lepus.unine.ch); the Benelux spider distribution maps (www.tuite.nl/iwg/Araneae/SpiBenelux/?species); the Czech Arachnological Society database (www.arachnology.cz/rad/araneae-1.html); the Atlas of the European Arachnids (www.atlas.arages.de) and previous iterations of what is currently the Iberian Arachnology Group database (www.sea-entomologia.org/gia/map/TableArachnoCP.html). Whenever available, information was also gathered to incorporate spatial error buffers around each record (e.g. when a locality was a park or town, the buffer became the possible error around a central point).

Experts and stakeholders were also consulted regarding particular species and specific threats or for their knowledge of study regions. Several colleagues also assisted in translating the literature and georeferencing historical records. Overall, those consulted were researchers, taxonomists, speleologists, conservationists, wildlife recorders, nature tourism guides, environmental NGOs, experts in the pet trade, law enforcement agents, park rangers, national environmental agencies and other government authorities, natural history museums or other research collections, local community leaders, among others. A number of those consulted are attributed in the acknowledgements of the species assessments (Seppälä et al. 2018a, b, c, d), and others will be mentioned in upcoming publications where their contributions were most valuable.

4.2.1.3. Species classification

The data thus gathered allowed us to assess each of the randomly selected species in our sample (N=200) against the five IUCN Red List criteria relating to extinction risk (IUCN 2012). These can be summarised in how available data can inform us about population size reduction - Criterion A; continued decline in geographic range, as Extent of Occurrence and/or Area of Occupancy - Criterion B; reduced number of mature individuals and population decline - Criteriona C and D/D1; very restricted range or small number of locations with a plausible future threat that could drive the taxon to extinction within a short time frame - Criterion D/D2; quantitative analysis to calculate the probability of extinction - Criterion E (IUCN Standards and Petitions Committee 2019).

The assessments of all these criteria allowed us to evaluate the placement of each of the analysed species in one of the IUCN Red List categories (IUCN 2012). These can be listed from the lower extinction risk to the higher as Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Extinct in the Wild (EW) and Extinct (EX). When the data available on the selected species did not allow for their category to be assessed, they were classified as Data Deficient (IUCN Standards and Petitions Committee 2019). Further details about the assessment approach itself have been made available in the method sections of the published species conservation profiles (Seppälä et al. 2018a, b, c, d).

4.2.1.4. Inferences and quality checks

IUCN’s guidelines for criteria A, C and E can be based on the analysis of a 10-year period or 3 generations, whichever is longer (up to a maximum of 100 years), while also being suitable to detect continuing decline under criterion B (IUCN Standards and Petitions Committee 2019). Once the generation length of most species within our sample was equal to or less than 3 years (Seppälä et al. 2018a, b, c, d), the longer period of 10 years was the one chosen to estimate or infer decline from habitat reduction analysis. For tree-dwelling species or those considered to be forest-dependent, population declines were in part inferred by the rates of deforestation recorded in the last 10 years, as made available by the Global Forest Watch portal (World Resources Institute 2014).

All species data, including threats, ecology, behaviour, declines and records, were reviewed by the first author (SH), which, in combination with the aforementioned experts (methods 2.1.2), allowed for knowledge gaps to be filled when necessary and for erroneous species records to be removed from the dataset. A number of records are also absent from the publicly shared dataset (Cardoso et al. 2019) to ensure the safety of those species and their populations (Lindenmayer & Scheele 2017).

4.2.1.5. Species mapping

The georeferenced records for each of the 200 species in our global sample (Cardoso et al. 2019) were gathered from their data repository in GBIF and subset according to the Red List category assigned to each species. From this dataset, we generated subsets of geographical records containing all DD species and all LC species, respectively.

We generated a density map of all records by aggregating the georeferenced occurrence into hexagons (using ggplot2's stat_binhex, bins=75) to reduce the geopolitical bias of aggregating the number of records per country, standardised by country area (Cardoso et al. 2019) and provide a more nuanced analysis of which regions within countries and across borders held the most records. We also generated two additional density maps using the same method, one for the aforementioned subset of DD species and one for the subset of LC species.

4.2.2 The sampled approach to the Red List Index

4.2.2.1. Equal-steps weights

Current RLI implementations use the equal-steps approach (Butchart et al. 2004; Butchart et al. 2007; Henriques et al. 2020) by assigning ordinal ranks to higher IUCN Red List categories. Following the equal-steps approach, species classified as Extinct (including Extinct in the Wild and Possibly Extinct) are assigned a weight equal to 5, CR =4, EN =3, VU =2, NT =1 and LC =0 (Butchart et al. 2004; Butchart et al. 2007). Species classified as DD do not contribute to the RLI value but were used to estimate confidence intervals (Butchart et al. 2010).

With this information, the RLI can be calculated as:

$$RLI_t = 1 - \frac{\sum_s W_c(t,s)}{W_{Ex} * N}$$

where the numerator is the sum of the weights of all non-DD Red List categories (W_c) across all species (s) in a given taxonomic group in a given year (t); in the denominator, W_{Ex} is the category weight for Extinct and N is the number of non-DD species in the target group being analysed (Butchart et al. 2007). In a sampled approach to the RLI, N is set as the number of non-DD species in the analysed sample (Henriques et al. 2020).

4.2.2.2. Bootstrap and extremes.

Bootstrapping has been proposed to search for statistical significance when comparing RLI among taxa or trends in time (Juslén et al. 2016). We also followed this approach, resampling all the species within our sample with non-parametric bootstrapping, because similarly to Juslén et al. (2016), an arithmetic analysis would also not be sufficient to show whether the resulting spider RLI was statistically different from the RLI value detected in other groups. Furthermore, non-parametric bootstrapping has been shown to correct estimate bias caused by replacements of missing data in a sample (such as species replacement; see methods 4.2.1.1), often improving power and efficiency (Mazen & Tong 2020). The confidence intervals of our bootstrap were calculated using the R package “boot” (Canty & Ripley 2020) based on the statistical functions of Davison and Hinkley (1997).

One of the most widely used methods for analysing missing data is multiple imputation (MI), which replaces missing values, such as the extinction risk weighting of DD species, with imputed values (Murray 2018). Therefore, additionally, to bootstrap, we also followed a commonly used MI approach by conducting a best-worst sensitivity analysis (Gamble & Hollis 2005) to calculate the extreme potential values assuming a best-case scenario, where all DD species are classified as Least Concern and are assigned a weight of 0 (upper bound) and a worst-case scenario, where all DD species are classified as Extinct and are assigned a weight of 5 (lower bound). This approach has commonly been used in RLI implementations (Baillie et al. 2010; United Nations 2015; IUCN 2020) and matches official guidelines of what uncertainty warrants a DD classification and what uncertainty warrants the application of the precautionary principle (IUCN Standards and Petitions Committee 2019). Subsequently, in this analysis, when implementing the RLI, N is no longer the number of non-DD species but encompasses all species in the targeted group (Baillie et al. 2010) or when implementing a sampled approach to the RLI, encompasses the complete sample size (here N=200).

4.2.3 Proportion of threatened species

The proportion of threatened species was calculated as [(Threatened) / (N - DD)], where *Threatened* is the number of spider species assessed as VU, EN or CR; N is the total number of species within the target group or the set sample size (here, total = 200) and DD was the number of spider species assessed as Data Deficient.

This approach assumes that DD species contain the same proportion of threatened species as the remaining non-DD species in the group (Richman et al. 2015). In some cases, this has been supported by predictive machine learning (Bland & Böhm 2016), although in others, predictive modelling suggests that the true extinction risk of DD species can increase the estimated proportion of threatened terrestrial mammals from 22% to 27% (Bland et al. 2015), and amphibians from 32% to 63% (Howard & Bickford 2014). Therefore, we also used a best-worst sensitivity analysis (as in section 4.2.2.2) by calculating the proportion of threatened species assuming that none of the DD species are not threatened [lower bound: (Threatened) / N] and assuming that all DD species are threatened [upper bound: (Threatened + DD) / N] as used in other implementations of the sRLI (e.g. Böhm et al. 2013), where the results solely based on non-DD species, described above, are defined as a complete case analysis (Gamble & Hollis 2005).

4.3. Results

Our random sample of global spider species (n=200) had 70.5% of species (n=141) classified as DD. Among the non-DD species, 6.8 % (n=4) were classified within a Threatened category (3 CR, 1 VU), and 93.2% of species were classified as LC (n = 55). The resulting RLI value was 0.9525, with bootstrapped confidence intervals between 0.9869 and 0.8837, with the possibility of being as low as 0.281, if all DD are Extinct, and as high as 0.986, if all DD are Least Concern (Figure 4.1). Overall, 6.7% of species were classified as threatened, with the possibility of being as low as 2% (if all DD are non-threatened) and as high as 72.5% (if all DD are threatened).

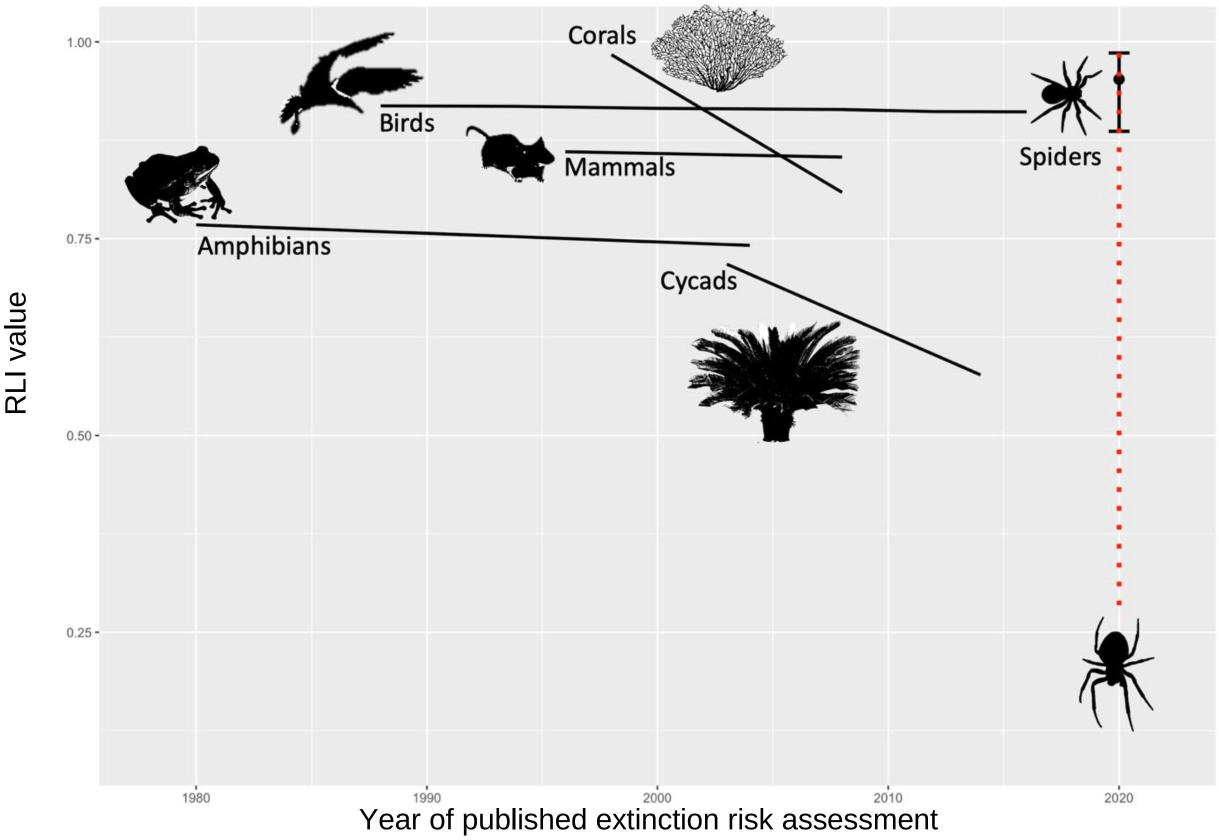

Figure 4.1 - Red List Index values of groups comprehensively assessed (amphibians, birds, cycads, corals and mammals) and the sampled RLI value of global spiders with the bootstrap confidence intervals (in black) and its best and worst case scenario (in red).

The overall species record map shows that this sample size can cover all major land masses (Figure 4.2A). However, it also presents sizeable gaps in hot deserts (e.g. North Africa), temperate deserts (e.g. Argentina), subtropical dry shrubland (e.g. Australia), the tundra grasslands as well as the Taiga boreal forest (e.g. Northern Russia), and tropical regions appear to be underrepresented, when compared to areas known to be less diverse, such as Europe or North America.

Records of LC species (Figure 4.2B) follow a very similar pattern to the pattern of records overall (Figure 4.2A), with a higher concentration of records in more studied regions (e.g. Europe, USA, Coastal Australia, South Africa and coastal Brazil).

Records of DD species present a distinct pattern (Figure 4.2C), with the highest number of these species being found within the borders of China (n=14), followed by the USA (N=11) and India (N=11). However, the USA and Mexico combined accounted for the region with the highest number of DD species (N=17). Obtaining better data for these North American species would represent a 12% reduction of DD species in our sample. Furthermore, records of DD species in those two countries are concentrated on the west coast (Figure 4.2C), making this the most effective region to be surveyed if efforts are ever undertaken to assess the true extinction risk of DD species in our sample.

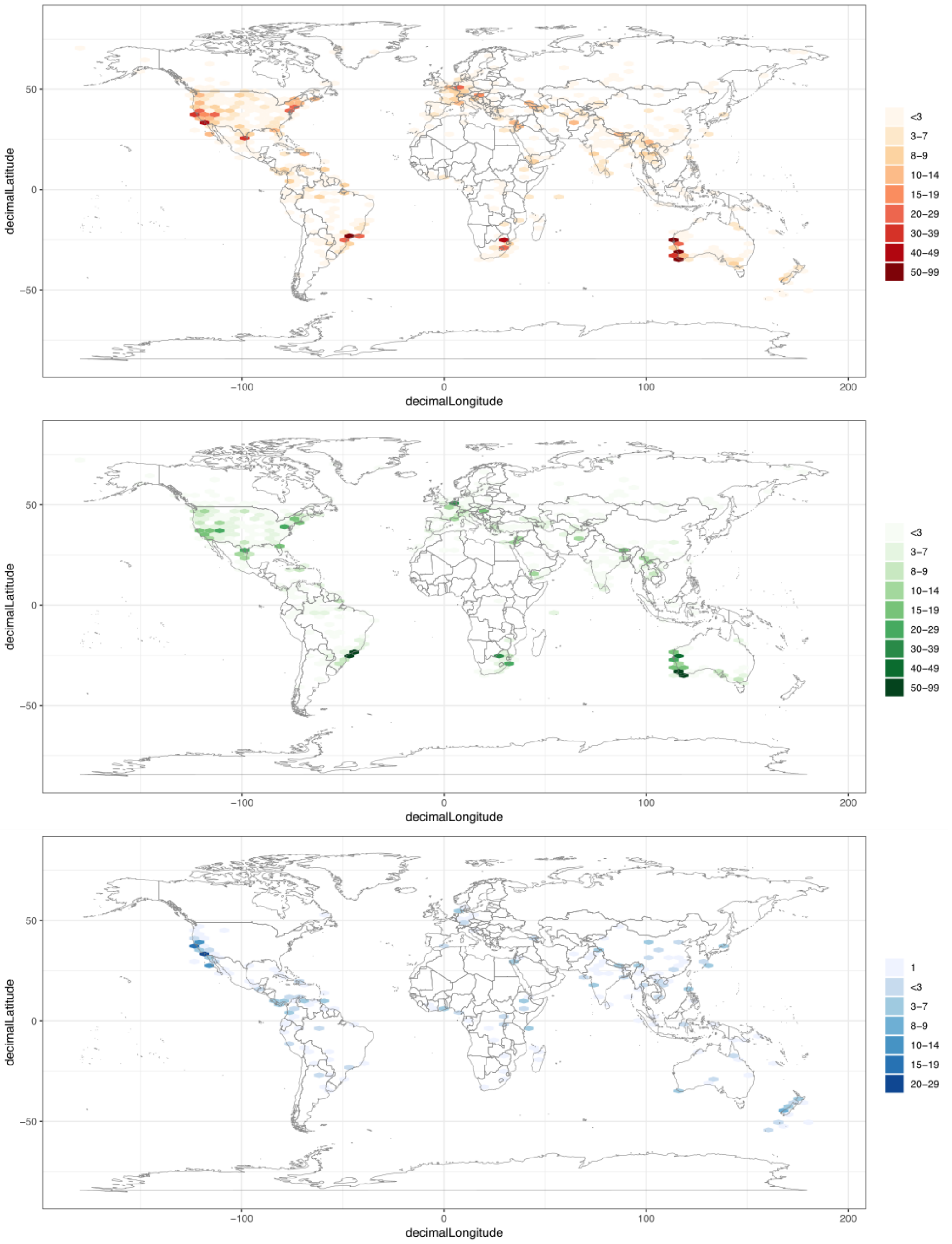

Figure 4.2 – Distribution of global records of (top) a random sample of 200 spider species (2,307 records); (middle) its subset of 55 Least Concern spider species (416 records); (bottom) its subset of 141 Data Deficient spider species (1,882 records).

4.2 Discussion

For the first time, we analyse the global extinction risk of spiders using the sampled approach to the Red List Index (Henriques et al. 2020). We showed that spiders might be among the taxonomic groups faring better, presenting the highest Red List Index value recorded for any group (RLI = 0.9525), or they could be the group faring worst, with the lowest RLI value so far recorded if all DD species are shown to be Extinct (Figure 4.1). The stark difference between RLI values in our sample, in a best-case (0.986) or a worst-case scenario (0.281), is a direct result of DD, which represented the vast majority of species in our sample (70.5%). However, we showed that even with this large percentage of poorly known species and relatively small sample sizes (N=200), a sampled approach could produce global coverage, capturing all the earth's land mass (Figure 4.2A). However, it is also important to note that geographical coverage is not the sole focus of conservation priorities (Gumbs et al. 2018; Owen et al. 2019; Gumbs et al. 2020), nor should it be the sole metric a sample aims to represent (Henriques et al. 2020).

Most of the regions unrepresented in our records were arid environments, either hot deserts (e.g. Sahara) or cold ones (e.g. Arctic Tundra), a pattern also found in other ecological datasets of global terrestrial biodiversity (Boakes et al. 2010; Martin et al. 2012). This data gap was potentially caused by the combination of two factors: lower species richness in those areas and the lack of surveys conducted in them (e.g. Durant et al. 2012). It is also important to note that despite having captured tropical areas in our sample, these records might underrepresent the high biodiversity richness these regions are known to have when compared to better-represented but lower diversity areas such as the US or Europe (Figure 4.2).

These biases are well understood, and within conservation science literature specifically, there is a strong geographical bias, with 40% of research published between 2011–2015 being carried out in the USA, Australia or the UK, whereas only 10% and 6% was carried out in the higher biodiversity-rich areas of Africa or South East Asia respectively (Di Marco et al. 2017). The ability to reasonably capture biogeographical realms with smaller sample sizes has also been supported by previous analyses (Henriques et al. 2020), and this reported bias in the literature strongly indicates that a larger sample size would likely not impact our coverage significantly but rather just increase the record density of the existing geographical bias.

The similarity in distribution pattern between the records of Least Concern (LC) species (Figure 4.2B) and the records of all species within our sample (Figure 4.2A) indicates that LC species are the main drivers of the geographical records within our sample as well as their gaps. This is not only expected from our dataset, where 93.2% of non-DD species were assessed as LC, but will also likely be the case for many groups implementing this approach in the future, as LC species are, by definition, those more likely to have, large geographical range and a sizeable number of records within it. Available geographical records for spiders also reveal that the areas with the highest density of LC records are located in developed regions such as the USA, Coastal Australia, South Africa, central Europe and coastal Brazil (Figure 4.2B). This is likely a reflection of where most research funding is focused (Wilson et al. 2016) and, hence, where species records have historically been made (Di Marco et al. 2017).

The distinct distribution pattern of Data Deficient (DD) species (Figure 4.2C) did not coincide with the least sampled regions of the globe (Boakes et al. 2010; Martin et al. 2012) nor with known biodiversity hotspots (Myers et al. 2000). Instead, areas of high concentration of DD appear to be in regions with considerable endemicity, where historically there was a substantial number of new species described (e.g. Western USA), but where subsequent research containing new records or analysis of species' range (e.g. biogeography, ecological or faunistic studies) was not done.

Geographical patterns of DD species are crucially important because, by definition, available data on these species are so poor that no conclusive Red List category can confidently be assigned to them, and DD has already been pinpointed as an ongoing dilemma for some of the most diverse regions of the world, like Africa (Siddig 2019). Therefore, given how costly increasing data availability can be, particularly when considering field surveys (Danielsen et al.

2005), determining how to make this process more cost-effective is key to optimising scarce research funding.

Although it is valuable to understand how DD species indicate an understudied biogeographical region, from a pragmatic standpoint, we considered countries' borders to be more indicative of potential logistical challenges when aiming to optimise species monitoring. We found China to be the country with the highest number of DD species within its borders (n=14). However, we also found that the highest concentration of DD species was in the western USA and Mexico (Figure 4.2C), which jointly represent 12% of all DD species in our sample (N=17). Therefore, we found this to be the most effective region to be surveyed when aiming to reduce the percentage of DD species in our sample.

Conducting target surveys in known and potential areas of DD species occurrence in China and neighbouring India could lead to an 18% reduction of DD species in our sample (N=25); however, surveys would be spread throughout a much larger area (Figure 4.2C) as the contiguous USA (officially named the conterminous United States) plus Mexico (9 636 491 km^2) is not only 25% smaller than contiguous China plus India (12 848 243 km^2) but North American records are mostly concentrated in the western region, which would cause sampling efforts to be substantially less resource intensive. Although it should be clear that if funds were available, surveying the specific habitats (known and predicted) of DD species in both regions would, of course, be a much better proposition, jointly reducing 30% of DD species from our sample. A distinct approach to potentially reduce DD from our sample is also discussed in Chapters 5 and 6, and several species assessments from this subset were re-analysed and included in supplementary material (selected and presented in alphabetical order).

Although the core reason for a DD assessment is because our knowledge of a species is so poor it could likely be LC or CR (or even EX), additionally, the IUCN guidelines recognise two tags for data deficiency, unknown provenance and when taxonomic uncertainty explains lack of information (IUCN Standards and Petitions Committee 2019). However, a comprehensive analysis of DD assessments has proposed further justification tags while recognising taxonomy as one of the main issues (Bland et al. 2017). Taxonomical uncertainty and consequent difficulties in disambiguating valid records from unreliable ones were clearly one of the main issues in our sample, affecting most DD and non-DD species (e.g. Eresidae, Seppälä et al. 2018a), and should therefore be a major focus of efforts to reduce the percentage of DD in our sample. We address taxonomic challenges for a complex case study of Eresidae (chapter 5) and propose additional paths to overcome them (chapter 6).

In an exploratory analysis of a small number of DD species in our sample (assessments presented in the Supplementary Material), we found the lack of taxonomic research to be the key issue where their original descriptions were the only available literature on these animals. The lack of taxonomic revision is largely responsible for the lack of further records, as available descriptions can be insufficient to allow further records to be made confidently, even if the species was observed. However, we also found unexplored potential from data sources such as museum records, which had not been previously analysed nor published, impacting our preliminary assessment (see supplementary material of chapter 6). Therefore, our results further support recent arguments about the importance of improving data sharing among researchers, particularly the value of information flow and improving global collation of spatial biodiversity data (Hochkirch et al. 2020).

However, increasing our knowledge of DD species towards understanding their extinction risk has not been a part of the sRLI protocol. Instead, the approach has been to select a wide enough sample of 1500 sp., aiming that the percentage of DD does not exceed 40% and allows the current target of 900 sp. non-DD to be met (Clausnitzer et al. 2009; Baillie et al. 2010; Böhm et al. 2013; Brummitt et al. 2015). This approach comes at a particularly high cost to the taxonomic groups that have the most to benefit from the sRLI (those that are understudied and highly speciose), as these are the groups that are also more likely to have high percentages of DD species, far exciding 40% (e.g. Minter 2011) where a 900 non DD sample can become an unfeasibly high target to meet. Therefore, increasing the number of analysed species can become one of the main obstacles to implementing the sRLI protocol, given that the larger the DD percentage, the larger the number of species one needs to analyse to capture the target non-DD set.

In our study group, assuming that the percentage of DD we found in our sample (70.5%) remains constant for larger samples of this group, the extinction risk of 678 spider species would have to be assessed in order to produce a sample of 200 non-DD species - the minimum sample which was recently shown to correctly detect RLI trend direction in non-avian groups with 95% accuracy (Henriques et al. 2020) and a sample size which could, under a 90% confidence, estimate the percentage of threatened species of global spiders within a 5% error from the truth (see chapter 3). However, a sample of 3051 spider species would have to be assessed to produce a 900 non-DD species dataset - the minimum sample size of the original sRLI protocol (Baillie et al. 2008), which was recently shown to detect a change in RLI slopes in all comprehensively assessed groups with 95% accuracy (Henriques et al. 2020).

Beyond the potential crippling resource demand necessary to address DD in this way, increasing the number of species in a random sample until the target non-DD set is found might also introduce bias: it is more likely to exclude species from areas where our scientific knowledge is low (Figure 4.2C) and increase the representation of species with sufficient data to be assessed, which are more likely to inhabit well-studied areas (Figure 4.2B). This, in turn, increases the probability of including LC species in a sample, as those will be the species with wider ranges that are more likely to be well represented in the species record. This is a non-trivial matter, as it might account for the high percentage of LC (72.5%) in our non-DD species set, which is the main driver for the high RLI value in our sample (0.953), the value which is used to track progress towards policy targets (chapter 1).

Recently, a growing number of publications have shed light on the drastic decline in insect populations, with studies recording a 75% decline in flying insect biomass in the past 30 years (Hallmann et al. 2017). This decline is even higher in particular insect groups, such as Dutch butterflies (van Strien et al. 2019), and does not appear to be limited to Europe, as it has increasingly been recorded in other parts of the world (Lister & Garcia 2018). Hence, it is very likely that spider species are also steeply declining under predictable cascading impacts on the food web (Hallmann et al. 2017). Despite a number of studies pointing to the complexities of measuring insect declines (Macgregor et al. 2019; Cardoso & Samways 2020; van Klink et al. 2020) and the dangers of an oversimplified narrative (Saunders et al. 2019; Didham et al. 2020), spider decline has in fact already been observed in the United Kingdom, the sole region where a monitoring scheme of this group has been in place (Outhwaite et al. 2020).

If several different lines of evidence, from habitat destruction and food web disruption to regional declines, indicate that a spider decline is ongoing, why does a random sample of global spiders have such a high RLI? It would be easy to dismiss this as an artefact of small sample size, but the distribution of records in our sample was global (Figure 4.2A), and the detected 70.5% of DD species point to a different challenge and deserve a different solution.

The current approach to randomly select a large number of species and exclude DD species to meet a target non-DD set makes the sRLI protocol unfeasibly large and hinders its application. Perhaps spiders present us with an opportunity to reconsider our approach to species conservation metrics, such as the sRLI: an opportunity to test our assumptions, where investing in targeted DD species surveys would allow us to measure the extinction risk of the species we know less about (Bland et al. 2015; Bland & Böhm 2016). This is a crucial endeavour that deserves to be made in a timely way before it is too late to measure extinction risk and we become solely able to measure where extinctions took place.

Conservation efforts have long been known to be biased towards charismatic species (Clark & May 2002), and despite notable efforts and the remarkable positive impacts of conservation action in preventing species extinction (Bolam et al. 2020), current policies do not appear to be reversing extinction trends or even halting them (IPBES 2019). Perhaps a paradigm shift towards understanding the extinction risk of DD species, even if it means moving from solely protecting the wildlife we love to protecting the wildlife we do not, could be a driver for positive change.

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Chapter 5

Importance of taxonomy for conservation and biodiversity indicators

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5.1. Taxonomy and the Sampled Red List Index

The sampled approach to the Red List Index (sRLI) was developed to measure conservation status trends by analysing a random sample of species from the target group (Baillie et al. 2008). Because of this, the sRLI metric is most useful in understudied and highly speciose groups (e.g. Minter 2011). However, the taxonomy of understudied and highly species-rich groups is also more likely to be unresolved, possibly contributing to species becoming excluded from the sample, as Not Evaluated (NE) or assessed as Data Deficient (DD). However, although taxonomic uncertainty appears to impact most taxonomic groups that have been analysed under a sRLI, how to approach taxonomic issues under this context appears not to have been uniformly addressed, as guidelines to what uncertainty warrants a species to be excluded, replaced or kept in a sample were never presented.

In this chapter, we discuss three taxonomic issues we found when implementing the sRLI for a megadiverse group, spiders (Chapter 4):
a) the importance of capturing updated taxonomic knowledge of a target group’s species;

b) the impact of taxonomy on the species inclusion or exclusion from a random sample;
c) the impact of taxonomic uncertainty in preventing or altering the placement of species in an extinction risk category.

5.1.1. Updated taxonomic knowledge

Since our implementation of the sRLI for spiders (2015), the global data set which was used to produce our sample (the World Spider Catalog) currently recognises 3,146 more species than it did at the time (World Spider Catalog 2016, 2020). With single publications describing 42 new species (Miglio et al. 2020), species numbers have been growing year by year: 887 new species were described in 2017 alone, 814 in 2018 and 834 in 2019 (World Spider Catalog 2020).

It is therefore not surprising that in the past decade alone, considered to be a feasible time period between the baseline sRLI and its reassessment (Henriques et al. 2020), the increase in the number of recognised spider species alone, 7721 sp. is higher than the number of all mammal species combined (Burgin et al. 2018). Because no conservation scientist would consider it reasonable to ignore the extinction risk of all mammals, it seems unreasonable to exclude a greater number of species from even being considered in a sampled approach because they were recently described.

Furthermore, new species are more likely to have remained undescribed to this day because they have small ranges, making them more likely to be classified as threatened (Meiri et al. 2018; IUCN 2020). Some newly described species, which are solely known from museum records, have already been considered likely extinct at the time of description (White et al. 2019) and became IUCN Red Listed as such (Critically Endangered possibly extinct) just the following year (Dulvy et al. 2020). Therefore, because current sRLI practices solely select from currently valid species, the effective exclusion of species described after the sample was made might introduce unforeseen biases.

To overcome the lack of inclusion of new species and the potential bias it might bring, we propose that sRLI guidelines should instruct those implementing this approach to update their sample every ten years by randomly sampling the same number of species from the global catalogue of the target taxa once, e.g. 1500 sp. under current recommendations (Baillie et al. 2008), and assess the extinction risk of all species within this new sample, that were described in the decade since. Under our proposal, these assessments would then replace a randomly selected set of species of the same size from the original sample and be used to calculate the RLI value.

In order to allow these species to contribute to the baseline RLI value (made ten years prior) and trend detection, we would encourage a retrospective assessment, as is currently done in comprehensively assessed groups, when taxonomic changes impact current species assessments. That impact is backcasted to be reflected in previous RLI (Butchart et al. 2004). In our own implementation of the sampled RLI of spiders, 9 of our 200 randomly selected species were described in the past decade, meaning they could not have been included if the protocol had been implemented a decade earlier. This represents 0.5% of the species sampled and can be a fair approximation of how many species we would add when reassessing our sample 10 years from now to detect the RLI trend.

5.1.1.1. Taxonomic replacements

When faced with taxonomic issues while selecting a random sample from a target group, what species will be assessed, need to be updated or are excluded and replaced has not been made clear in the literature to the best of our knowledge and seems to rely on individual researchers' own interpretation. However, this might introduce attrition bias in a sample (Ghanem et al. 2020; Taylor et al. 2020) and is therefore important to address in a systematic and transparent way.

A recent sRLI for freshwater molluscs found 13 species of their randomly selected species had to be replaced as they were synonymised with other species already included in the sample (Böhm et al. 2020). A sampled approach of several groups of plants found that 172 species of their randomly selected sample were affected by homotypic or heterotypic synonyms (Brummitt et al. 2015a; Brummitt et al. 2015b). These examples show how even small samples are prone to considerable taxonomic change in relatively short amounts of time, from species selection to species assessment.

In our own implementation of the sRLI for spiders (chapter 4), the species *Plexippus gajbei,* which was considered a valid species at the time of sampling (2015), became regarded as a synonym of *Carrhotus viduus* the following year (Caleb 2016). This, in turn, led to the inclusion of *Carrhotus viduus* in our sample, with considerable implication to the range and extinction risk assessment, as *Plexippus gajbei* was solely reported for a single site in the Western Ghats of southern India, whereas *Carrhotus viduus* occurs from Iran to India and China (World Spider Catalog 2020). The species *Tobias paraguayensis*, solely recorded from Western Brazil and neighbouring Paraguay, was also selected to be included in our sample but was soon after considered a synonym of *Epicadus trituberculatus* (Machado et al. 2017), recorded from Mexico to Argentina (World Spider Catalog 2020).

These replacements might appear simple to justify but are impactful in this context, as wider ranging species are more likely to be assessed under lower threat categories (IUCN Standards and Petitions Committee 2019). The sRLI is a rather unique example in sampled research, where independent taxonomic work can lead to a participant species being replaced by a study subject with significantly different characteristics. It is, therefore, perhaps not surprising that without clear guidelines, not all of those who implemented the sRLI dealt with taxonomic issues in the same way.

In the sRLI for Monocot plants, when synonyms lead to a species replacement which was considered likely to produce a different assessment, the species names were not taxonomically updated but replaced by other species names selected at random (Brummitt et al. 2015b). This inclusion of additional randomly selected species from the target taxonomic group to replace the ones excluded would fit what has been called a refreshment approach (Mazen & Tong 2020). In the sRLI for dragonflies, when replacement was considered necessary at least some species were replaced by another from the same realm and family, from an additional set of 500 randomly selected species (Clausnitzer et al. 2009). This replacement approach, where additional species are selected based on auxiliary variables, such as realm and family, that are considered to potentially explain the missingness in the original set, would fit what has been called a replacement approach (Mazen & Tong 2020).

The fact that the sRLI has been conducted differently reduces reproducibility and is likely to impair the value of across taxa comparisons. Because taxonomic groups that are analysed under a sRLI are likely to come across similar issues, we propose that guidelines instruct those implementing this protocol to standardise the replacement method used and to encourage clear reporting of what the original species list, before any replacement, was. This list would not only increase transparency, but if synonyms are ever proven incorrect and reversed, or if any future taxonomic work impacts any of the species replaced, it will allow for the species names that were originally randomly selected to continue to be the ones informing the sRLI.

This is not an unreasonable scenario, *Plexippus yinae* a species solely recorded from China, was randomly selected to incorporate our sample (2015), but just a year later was synonymised with *Plexippus clemens* (Caleb 2016), a species recorded from Mediterranean, African and Asian countries (Seppälä et al. 2018c). However, by then, it had become a synonym of *Plexippus tectonicus* (Prószyński 2017), a species solely recorded from Israel and China (World Spider Catalog 2020). Despite not being followed by all researchers (e.g. Peng 2020), this synonym is currently recognised by what we consider the authoritative world catalogue (World Spider Catalog 2020). It is therefore important that each taxonomic group clearly sets their authoritative taxonomic source and ensures the sampled species are not only taxonomically updated at the time of the sRLI implementation but are continuously updated at least every decade.

5.1.1.3. Taxonomic uncertainty and extinction risk assessments

Not only species replaced but also those kept can present issues that are important to address. In our sample (chapter 4), *Hogna exsiccatella* was randomly selected and was included in our analysis (Seppälä et al. 2018b), for which solely one female specimen has been recorded from an unspecified date prior to 1916, recorded from an unspecified locality probably in Guatemala, although the author added a question mark to the country of origin (Strand 1916). When this specimen was analysed over a century ago, it was already considered to be in poor preservation condition and probably lacking several distinctive features such as colouration (Strand 1916). Furthermore, the solely known type specimen appears to have become completely destroyed since or became lost (Nentwig pers. comm.).

The author who originally reported on *Hogna exsiccatella*, Embrik Strand, described 382 spider taxa between 1906 and 1917, a considerable portion of which is no longer considered valid, with 64 species declared *nomina dubia* and 57 species now synonymous with other species (Nentwig et al. 2020). *Hogna exsiccatella* was not part of this analysis (Nentwig et al. 2020) and remains an accepted species (World Spider Catalog 2020). However, with the available description, without a single scientific illustration and the loss of the type material, it is impossible to recognise this species, even if it was found (Jordán pers. comm.). Therefore, its DD status, even if tagged as due to “taxonomy uncertainty” or “uncertain provenance” (IUCN Standards and Petitions Committee 2019), is somewhat misleading since this uncertainty will likely never get resolved (Bland et al. 2017) and consequently cannot contribute to detect a trend in a sampled approach to the RLI (Baillie et al. 2008).

Historically, several species have been removed from the IUCN Red List due to doubtful taxonomic validity (Bland et al. 2017). Because we proposed that conservationists should aim at assessing the extinction risk of all the species in a sample rather than pursuing a non-DD set (see chapter 4), we consider that when no further data can be gathered to inform a species extinction risk, that such species should be removed and replaced in a sampled approach to the Red List Index, while leaving a clear register of which species have been excluded and why.

5.1.1.4. Impacts of taxonomy in DD and non-DD species

The lack of taxonomic research as an impediment to species extinction risk assessment and the consequent impact on Data Deficient classifications, has long been recognised by the IUCN Red List (IUCN Standards and Petitions

Committee 2019) and is broadly understood to be a significant problem in conservation (Butchart & Bird 2010; Bland et al. 2012; Trindade-Filho et al. 2012; Morais et al. 2013; Bland 2014; Howard & Bickford 2014; Bland et al. 2015; Nori & Loyola 2015; Bland & Collen 2016; Bland & Böhm 2016; Bland et al. 2017; Braulik et al. 2018; Scherz et al. 2019; Xing et al. 2019; Hochkirch et al. 2020). However, not only DD species are affected by taxonomical issues.

The iconic ladybird spider *Eresus kollari*, protected in several European countries (Milano *et al.* in press), was randomly selected to be part of the spider sRLI (Seppälä et al. 2018a). Although it was currently not possible to disambiguate which geographical records under this species name do, in fact, belong to this species, it was not excluded from our sample (Seppälä et al. 2018a) and due to its relatively wide range was assessed as Least Concern.

Analysis of this species' complex taxonomic history not only revealed that it contained several valid species that had erroneously been synonymised under this name (when they were in fact distinct), but also that *Eresus kollari* itself is a junior synonym of *Eresus cinnaberinus*. Therefore, this group presents a remarkable study case as it covers a range of taxonomic issues that other species in other groups might also encounter, namely what is often called species lumping or splitting. Our analysis of this group perfectly illustrates how selection criteria, determining which species are excluded or remain in an sRLI under taxonomic uncertainty, matters. As a single randomly chosen species, *E. kollari*, when comprehensively reviewed (including in-depth analysis of all the literature in five different languages, fieldwork across most of the reported range, from Portugal to Kyrgyzstan, and extensive analysis of museological specimens, from the UK to Iran) contained at least five valid species, with distinct ranges and affected by considerably different threats, *Eresus niger, Eresus cinnaberinus, Eresus fulvus, Eresus tristis* and *Eresus illustris*. We present the first steps towards a taxonomic analysis of *Eresus* and the closely related *Loureedia* genus in the following section of this chapter, where we also describe a new species for science dedicated to our friend and colleague Ben Collen (12 February 1978 – 19 May 2018).

5.2. First records of *Loureedia* (Araneae, Eresidae) from Europe, with the description of a new species and a survey of the genus

Abstract

The genus *Loureedia* Miller, Griswold, Scharff, Řezáč, Szűts & Marhabaie 2012 is recorded for Europe for the first time, with the description of a new species *L. colleni* sp. N. from southeast Spain, differing markedly from other species of this genus in biogeography, genital morphology and the striking white dorsal pattern of the males. *Eresus albopictus* and *E. lucasi* are considered distinct species, and we transfer the latter to *Loureedia* as *L. lucasi* comb. N., species which we propose as a senior synonym of *L. maroccana,* n. syn. We, therefore, recognise three valid species for the genus *L. colleni* sp. N., *L. lucasi* and *L. annulipes*, the latter of which we recorded as occurring in Libya for the first time while reporting this genus for Iran for the first time, considerably extending its current known range.

This work is dedicated to Ben Collen's daughter, Ottilie Collen.

5.2.1. Introduction

Velvet spiders (family Eresidae) occur in a variety of habitats in the Palearctic, Afrotropical, Indomalayan, and Neotropical regions (Jocqué et al. 2006; Miller et al. 2012; World Spider Catalog 2018). The family includes nine genera and almost one hundred species, most with African distribution (World Spider Catalog 2018) and only one in the New World (Kraus & Kraus 1992). Despite their wide distribution (World Spider Catalog 2018), appealing appearance, recognised conservation value (Franc 2000; Řezáč et al. 2018; Seppälä et al. 2018a), and the fascination they have instilled in scientists for centuries (Schaeffer 1767), our knowledge on this family is scarce, and even the taxonomy of the presumably well studied Central European species has only recently been clarified, and much remains to be done for it to be fully understood (Řezáč et al. 2008; Pérez-Zarcos & Sánchez-Piñero 2016). It is, therefore, not surprising that an understudied biodiversity hotspot, such as the Iberian Peninsula, still contains several undescribed species (Cardoso & Morano 2010).

According to the website “Araneae. Spiders of Europe” (Nentwig et al. 2018) the eresid species currently recorded in Europe belong to four genera: *Eresus* Walckenaer, 1805; *Adonea* Simon, 1873; *Stegodyphus* Simon, 1873; and *Loureedia*, Miller et al., 2012 (Gál et al. 2016; Nentwig et al. 2018). This revision work aims to produce a revision of the latter, as its records in Europe were never confirmed or published and were misidentified.

5.2.2. Methods

5.2.2.1. Taxonomic analysis

Specimens were analysed using a Zeiss SV11 stereo-microscope and a Canon EOS 450D camera. Extended depth of field images were obtained by stacking photos using Helicon Focus v. 5.2 software, which assisted in preparing the illustrations at the Sackler Biodiversity Imaging Lab., Natural History Museum, London.

A male specimen was critical point dried, then mounted on stubs or round-headed rivets using a combination of white glue, nail polish, and adhesive copper or aluminium tape. They were sputter coated with platinum-palladium and scanned with a Hitachi S-510 scanning electron microscope at the “Centro de Instrumentación Científica”, Universidad de Granada.

Morphological terminology follows Miller et al. (2012) and has been adapted to describe the fine morphological structures of the male pedipalp.

5.2.2.2. Potential distribution mapping

There are numerous methods for predicting species distributions based on ecological niche modelling, often with attempts to determine the environmental space occupied by a study species and to produce maps of these preferences in geographical space as potential species distributions (e.g. Guisan & Zimmermann 2000; Scott et al. 2002).

In our study, the hypothetical distribution of species, the range of which included North Africa and the Middle East (*L. annulipes* and *L. lucasi*), was adapted with the recently revised semi-arid climatic zones of the Köppen-Geiger climate classification (Kottek et al. 2006; Rubel & Kottek 2010; Rubel et al. 2017, available online at http://koeppen-geiger.vu-wien.ac.at).

The species distribution model, the range of which was mainland Spain (*L. colleni* sp. N.), was tested by logistic regression, making adjustments to expected binomial distributions of the environmental variables in sampled sites. We used Generalized Linear Models (GLMs), a standard and well-known method extensively used in ecology and closely related fields (see basics of GLM, e.g. in Dobson 1990; Crawley 1993; Dunteman & Ho 2006).

This approach has proved reliable when considerable presence-absence data are available (which we were able to gather for mainland Spain), as it allows to reduce the impact of false absences in the potential distribution. Presence-absence data of *L. colleni* sp. N. was gathered by generating a sampling network of 350 localities in the South of the Iberian Peninsula. These localities were randomly arranged within the main altitude and aridity gradients from the records of this region.

A spatial database containing the environmental characteristics of these sampled sites was generated, represented by the values of different bioclimatic variables under a 1 km^2 spatial resolution (UTM 1x1 km grid). We used 19 bioclimatic variables derived from temperature and rainfall values as provided by the Worldclim database (Hijmans et al. 2005). Altitude and other ecologically relevant variables such as steepness and cardinal directions of slopes and hillsides were measured using Geographic Information System (GIS) software and using Digital Elevation Model (DEM) with a 90 x 90 m spatial resolution of the Iberian Peninsula, as part of NASA's Shuttle Radar Topography Mission, SRTM v.1, (Farr & Kobrick 2000), and curated by the CGIAR-Consortium for Spatial Science (available online at *https://srtm.csi.cgiar.org*).

The selection process of the most significant models was carried out in R v. 3.3.2 (R Core Team 2014) using the ‘glm’ function to analyse the link between the different variables and the presence/absence of the species. GLM was used in a binomial function with the script “glm (formula, family = ‘binomial’, (link = ‘logit’), data = ‘data source’)”. Each variable was formulated, tested and contrasted against all other options using a step-by-step procedure with automated protocols for the different formulas in their linear and quadratic forms. Data transformation was considered when a normal distribution was not present in the raw data of independent variables.

The GIS software used in the cartographic representations was GRASS (Grass

Development Team 2016)” and “QGIS (QGIS Development Team 2016). DEM was also used to create a shaded 3D mapping of background (self-developed colour palette). On this background mapping, coloured vectorial polygons were added, representing the cumulative probability of occurrences of *L. colleni* sp. N. Dark blue areas representing 90% of localities where *L. colleni* sp. N. potentially occurs. Light blue represents the areas where the probability of finding populations of this species is between 5 and 10%. The section is coloured by both blue tones, hence representing an area where at least 95% of localities with this species are predicted to be found. Therefore, our models predict that at most 5% of *L. colleni* sp. N. localities are found outside the coloured areas, likely in habitats with poorly suited ecological niches, where observations might still be made in the future but where the conditions for the species to thrive are poor.

The Mediterranean basin map was made under the Times Projection System (WGS84 Datum), while the inset with the species records from the Southeast Iberian Peninsula is projected in the UTM System (Zone 30, WGS84 Datum).

No exact coordinates are provided for the locality data, and only 10 x 10 km spatial resolution in Military Grid Reference System – MGRS (UTM grid) are given, except when that data has already been reported in previous publications. This was done once we feared that, if disclosed, exact localities would endanger those populations by exposing them to the illegal pet trade (Lindenmayer & Scheele 2017). We understand the importance of open data and reproducible science, and we believe our approach achieves a reasonable compromise between transparency and species conservation concerns. We would welcome a taxonomical data management system where sensitive data can be safely stored and only made available upon request by verifiable, trustworthy entities that adhere to data privacy and safety protocols.

5.2.2.3. Biological Data

The phenology of *L. colleni* sp. N. was inferred from photographic records of males and the collection dates of the analysed material.

Diet and microhabitat were inferred from the observation of nest content and their location, which were validated as belonging to *Loureedia* sp. (rather than nests of other eresid genera) by collecting and identifying the individual specimens found inside the nest. No abandoned nests were therefore used for this analysis.

5.2.2.4. Abbreviations

HUJ – Hebrew University of Jerusalem.
NHM – National Museum of Natural History (London).
MCNB – Museo de Ciencias Naturales (Barcelona).
MNHN – Muséum National d'Histoire Naturelle (Paris).
MNCN – Museo Nacional de Ciencias Naturales (Madrid).
Some specimens used for this research are deposited in the personal collection of:
JKPC – Johan van Keer private collection.
JMC – Jesús Miñano private collection.
RPC – Milan Řezáč private collection.
SHPC – Sérgio Henriques private collection.

5.2.3 Results

Loureedia Miller, Griswold, Scharff, Řezáč, Szűts & Marhabaie, 2012
urn:lsid:zoobank.org:act:5FEC8D28-5F6F-4E58-A5C2-5EEBD35B0090
http://species-id.net/wiki/Loureedia

***Loureedia lucasi* (Simon, 1873)**
Eresus lucasi Simon, 1873: 353, pl. 10, f. 8-9. Comb. Nov.
Loureedia maroccana Gál, Kovács, Bagyó, Vári & Prazsák, 2016: 13, Figure 5.**Error! Unknown switch argument.**Figure 5.**Error! Unknown switch argument.** Syn. Nov.

DISTRIBUTION: Algeria: 1 ♂ 1 ♀ Oran [no date] Lucas, MNHN, type series; 1 ♂ Lalla-Maghnia [no date] Lucas (not examined, MNHN), type series. **Morocco**: 1 ♂, Sidi Boukhalkhal, 04 September 2013, J. Gál; 2 ♂, Sidi Boukhalkhal, 28 September 2015, R. Bagyó (not seen but analysed via depictions in Gál et al. 2016).

TAXONOMIC HISTORY: This species was originally described as *E. lucasi* Simon, 1873 and was later synonymised with *Eresus albopictus* (Simon, 1910), but our analysis has proven this synonym not to be accurate as the holotype of *E. albopictus* clearly belongs to the *Eresus* genus, whereas *E. lucasi* possesses *Loureedia* features (Figure 5.**Error! Unknown switch argument.** a-f).

The first recorded specimens of *L. lucasi* were a male and female from Algeria collected by Lucas in the outskirts of Oran and a second male from Lalla-Maghnia, near the Algerian border with Morocco (Simon 1873).

Several other specimens reported as *E. albopictus* that might belong to *L. lucasi* were reported from Orléansville and Daya in Algeria (collected by L. Bedel), from the North African enclave of Melilla (collected by Arias) and from Morocco, when de la Escalera collected conspecific male and female from Essaouira (Simon 1910). However, none of these specimens is currently available for analysis, and it is yet unclear which of these records may have been misidentified L. lucasi specimens and which truly belong to *E. albopictus*.

The taxonomic identity of these records and the revised distribution of *E. albopictus* is part of an upcoming revision of the *Eresus* genus and was considered to be beyond the scope of this publication, which is aimed at addressing the *Loureedia* genus.

SPECIES DESCRIPTION: *L. lucasi* males were originally illustrated and described as having a highly elevated and convex, carmine red cephalothorax and abdomen with longitudinal bands of red (Simon 1873). Characters which also match *L. annulipes* (Miller et al. 2012).

However similar, the taxonomic distinction between these two species, *L. annulipes* and *L. lucasi* (as *L. maroccana*), have already been comprehensively explored (Gál et al. 2016). Therefore, here we will solely focus on recording specimens from the *L. lucasi* type series, particularly the male specimen (Figure 5.**Error! Unknown switch argument.**a-d), in order to validate the genus transfer of *E. lucasi* to *Loureedia* (comb.nov.) and its synonym with *L. maroccana* (syn. Nov.), recording the most relevant characters of the females of this species for the first time (Figure

5.**Error! Unknown switch argument.**e-f). For more detailed information on the traits of this species, see the original description of *E. lucasi* (Simon, 1873) and the comprehensive description of *L. maroccana* (Gál *et al*., 2016).

***Loureedia annulipes* (Lucas, 1857)**
http://species-id.net/wiki/Loureedia_annulipes

DISTRIBUTION: Egypt: 1 ♂, Alexandrie [no date] (AR836, original code 471, original label written by Simon, MNHN, syntypes); **Israel**: 1 ♂, Haluqim Ridge (n. Sede Boqer), 17 November 1990, Y. Lubin (MR008, HUJ not seen but analysed via depictions in Miller et al. 2012); 1 ♂, Negev, Nitzana [Nitzanna] village, 1 October 2004 (MR018, HUJ, not seen but analysed via depictions in Miller et al. 2012); 1 ♀, Negev, Wadi Mashash, 4 December 2004, J. Kral (MR019, RPC); **Libya**: 1 ♂, Benghazi District, 1984 (labelled as: 84) D. Pearson (NHM).

TAXONOMIC HISTORY: Previous publications mentioned that *Eresus annulipes* was first recorded as a female from Rio de Janeiro, while it was later noted that the type specimen is in fact labelled as *Patria Ignota* (Miller et al. 2012).
Although we do not dispute that the lack of location in the specimen's label [*Patria ignota*] disagrees with the locality recorded in the original description [Rio de Janeiro], our analysis of the original publication also revealed that the holotype was in fact not a female but indeed a colourful male, as made clear in Lucas (1857)'s quote: "l'organe excitateur est rougeatre, globuliforme, et armé à son extrémité d'un crochet corné, noir, bi-épineux." Which accurately describes the male palp reported for *L. annulipes* (Miller et al. 2012).
Perhaps what originated the confusion on the gender of the specimen mentioned in this description was the sentence, "Cette jolie espèce, I je ne connais que le male" [This beautiful species, of which I know only the male]. Perhaps the French w"''Idont" has been misinterpreted with the "ngl's" "don't", causing some readers to infer that the male was not analysed.

SPECIES DESCRIPTION: *L. annulipes* was first illustrated with considerable detail for the cover of "*Initia'ion à l'étude systématique des araignées*" (Ledoux & Canard 1991), descriptive illustrations and measurements had also been published from Egyptian type material originally identified as *E. semicanus* (El-Hennawy 2004) and both males and females were comprehensively described and photographed when the genus was erected to hold this species (Miller et al. 2012).

FURTHER NOTES ON *LOUREEDIA.* For clarity in communication, in this publication, we informally distinguish this genus into two morphological groups. One with the species currently known from North Africa and the Middle East with brightly red males (*L. annulipes* and *L. lucasi*), which we call the *L. annulipes* group, and species from the Western Mediterranean with white patterned males (currently with only one species: *L. colleni* sp. n.), which we call the *L. colleni* sp. n. group. We present further information on the first group.

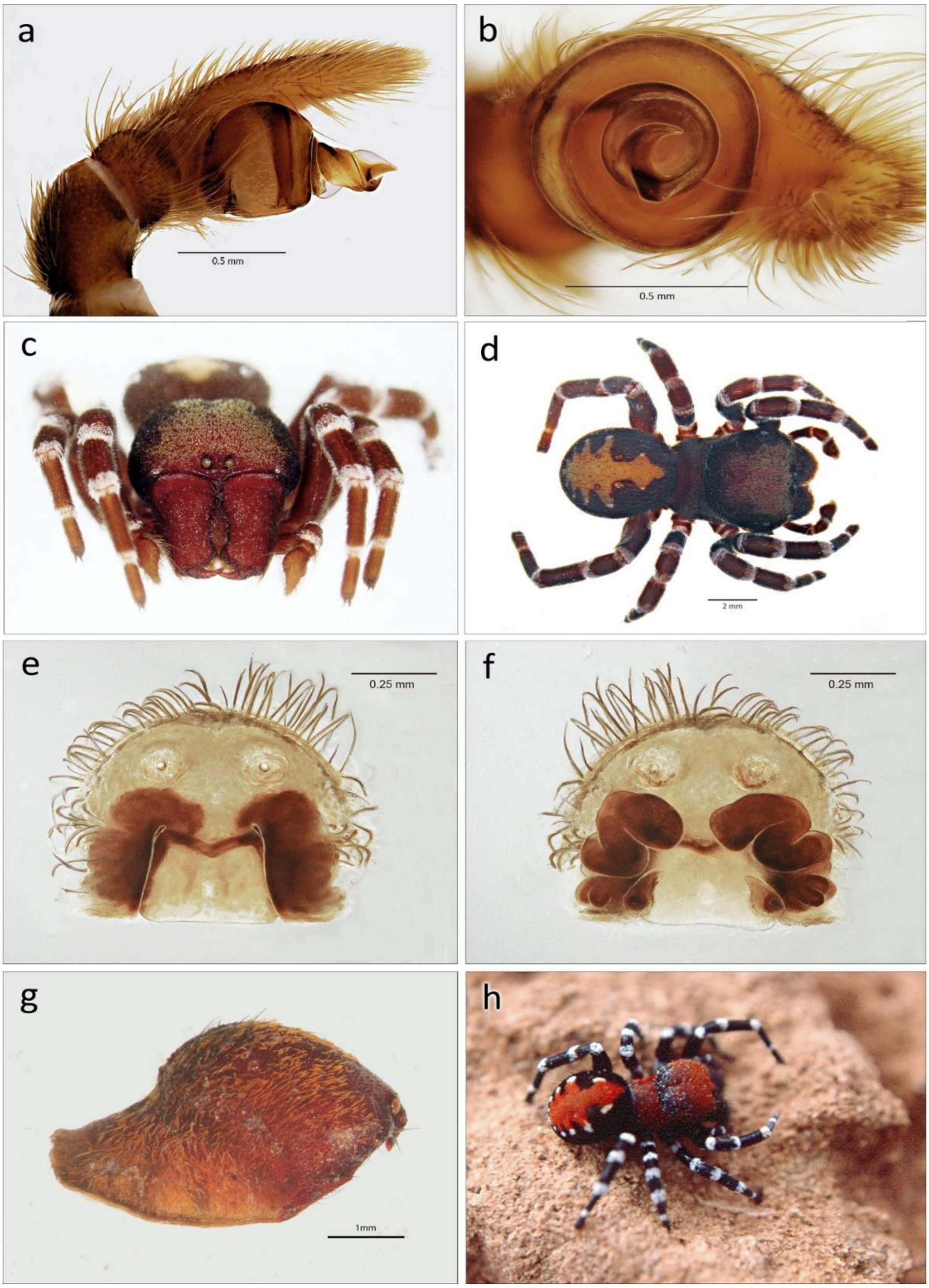

Figure 5.**Error! Unknown switch argument.** - *Loureedia lucasi*: [**a**] Lateral view of the male pedipalp of *Loureedia lucasi*; [**b**] Axial view of the male bulb of *L. lucasi* (type series); [**c**] Frontal view of a male *L. lucasi* (type series); [**d**] Dorsal view of a male *L. lucasi* (type series); [**e**] Ventral view of the female genitalia of *L. lucasi* (type series); [**f**] Dorsal view of the female genitalia of *L. lucasi* (type series); [**g**] Lateral view of a male carapace of *Loureedia* cf. *lucasi* found in a female's nest (Agadir-Ida Ou Tanane,

province, Morocco); [**h**] Male *L*oureedia cf. *lucasi* (Kénitra Province, Morocco) Photo by Luis García-Cardenete.

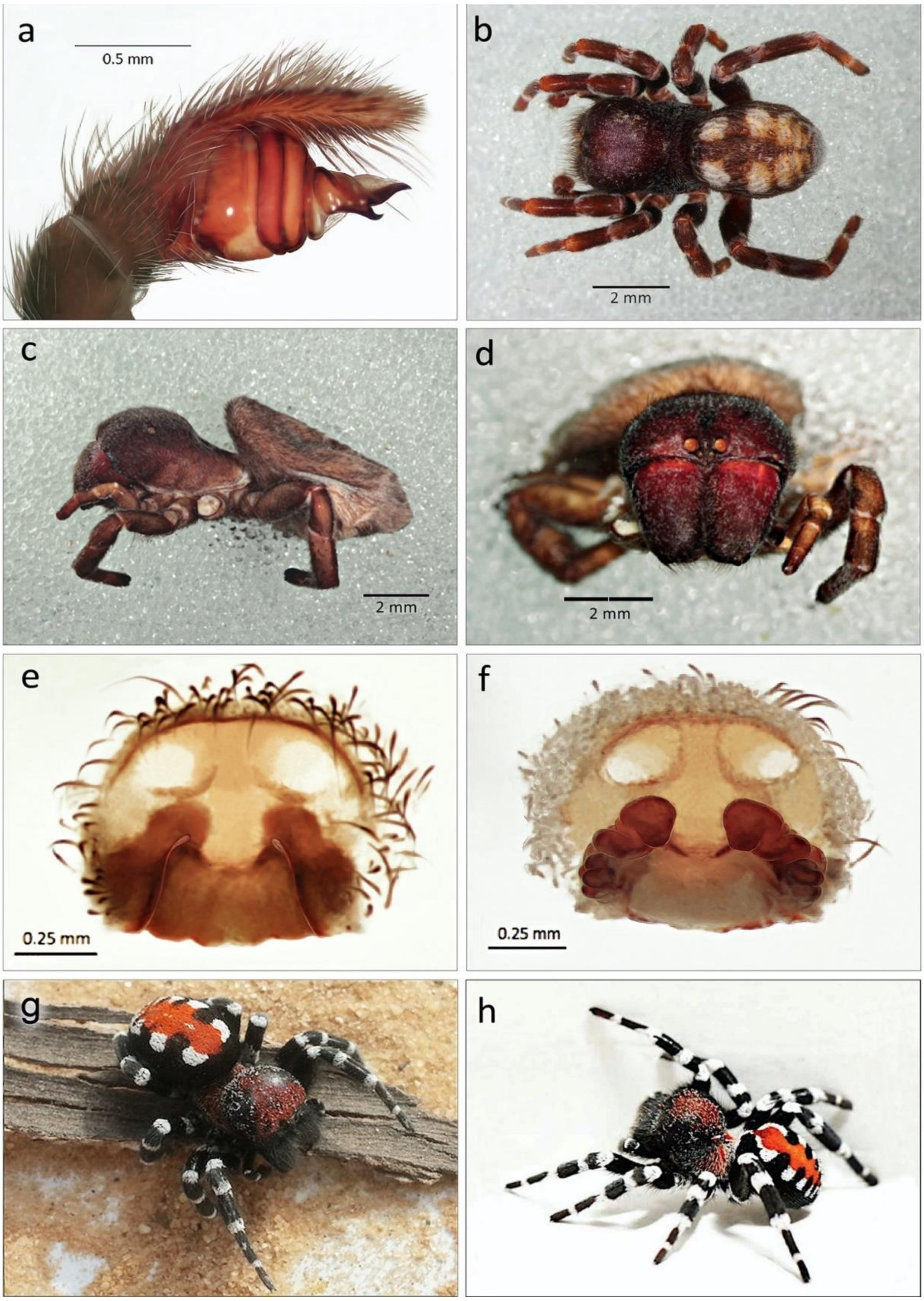

Figure 5.**Error! Unknown switch argument.** - *Loureedia annulipes*: [**a**] Lateral view of a male palp of *L. annulipes* (Benghazi, Libya); [**b**] Dorsal view of a male *Loureedia annulipes* (Benghazi, Libya); [**c**] Lateral view of a female *L. annulipes* (Israel); [**d**] Frontal view of a female *L. annulipes* (Israel); [**e**] Ventral view of the genitalia of female *L.*

annulipes (Israel); [**f**] Dorsal view of the genitalia of female reproductive organs *L. annulipes* (Israel); [**g**] Dorsal view of male *Loureedia* sp. from Zawiya District, Libya (Photo by Osama O. Etewish); [**h**] Dorsal view of male *Loureedia* sp. from Tehran

GEOGRAPHICAL DISTRIBUTION. Thus far, the *L. annulipes* group is only known from the South and Eastern Mediterranean (Algeria, Israel, Egypt, and Tunisia). In this work, we report the occurrence of *L. annulipes* in Libya for the first time (Figure 5.**Error! Unknown switch argument.**a,b). Also noting that similar specimens (Figure 5.**Error! Unknown switch argument.**f) pertaining to *L. annulipes* or *L. lucasi* have been sighted at several points along the Libyan coast (Osama O. Etewish pers. comm.).

We also believe it is worth reporting on the remarkable photographic records from Iran that clearly bear features from the *L. annulipes* group (Figure 5.**Error! Unknown switch argument.**h):

Iran: 1 ♂, Tehran Province, October 2015, Amir Hossein Bolhari (photographic record), ♂, Tehran Province, November 2016, Alireza Zamani (photographic record).

These records extend the genus range considerably eastwards. Photos of other specimens with similar features from a distinct, independent source in the country were also observed, providing compelling evidence that the genus is likely native to the country. However, human introduction cannot be excluded. It is likely that these Iranian records belong to a distinct and undescribed *Loureedia* species. Unfortunately, the photographed specimen was not collected, and preserved specimens from other records have been lost or not been made available.

BEHAVIOUR: Identifiable parts of the bright red males, including a carapace (Figure 5.**Error! Unknown switch argument.**g), have been found in a female‘s nest, possibly accounting for post-mating cannibalism.

Loureedia colleni **Henriques, Miñano & Pérez-Zarcos sp. n.**

HOLOTYPE: SPAIN: Andalucia: ***Granada province*****:** Granada municipality, 820m, 1 ♂, manual collection, 10 October 2010, (deposited –t MNCN - 20.02/19374), Carlos Jerez del Valle Leg. [30SVG51].

PARATYPES: SPAIN: Andalucia: ***Almeria province***: Almeria municipality, 27m, 1 ♂, manual collection, 10 October 2018, MNCN 20.02/19375, Francisco Rodríguez leg. [30SWF47]; Vicar 82 m 1 ♀, manual collection, 26 March 2014, MNCN 20.02/19376, Francisco Rodríguez leg. [30SWF37]; Vicar 82 m 1 ♀, manual collection, 11 April 2014, MNCN 20.02/ 19377, Francisco Rodríguez leg. [30SWF37]; Sorbas, 370 m, 1 – (MCNB - MZB 2018-0674) + 1 – (MCNB - MZB 2018-0674), pitfall, 06 September to 05 December 2017, (MCNB), J. Miñano leg. [30SXH16]; Murcia Region: ***Murcia province***: Fortuna, 120 m, 1 ♂ + 1 ♀, manual collection, 26 April 2004, (MNHN), J. Miñano leg. [30SXH62]; Abanilla, 155 m, 2 ♂, pitfall, 01-30 October 2003, (MNHN), J. Miñano leg. [30SXH87].

ADDITIONAL SPECIMENS: SPAIN: Andalucia: ***Almería province***: Albox, 770 m, 1 ♂, manual collection, 08 November 2010, (JMC), J. Miñano leg. [30SXG13]; Cabo de Gata, 12 m, 1 ♂, pitfall, 10-17 May 2004, (JMC), A. Aguirre leg. [30SWF67]; Cabo de Gata, 6 m, 1 ♂, wandering, 01 April 2009, (JMC), J. Miñano leg. [30SWF49]; Cuevas de Almanzora, 135 m, 1 ♀, pitfall, 07-28 April 2011, (JMC), J. Miñano leg. [30SXH57]; El Ejido, 15 m, 1 ♂, wandering, 14 April 2006, (JMC), J. Miñano leg. [30SWF37]; Nijar, 40 m, 1 ♂, under stone, 19 February 2010, (JMC), J. Miñano leg. [30SXG64]; Tabernas, 300 m, 2 ♂, pitfall, 11 March to 05 June 2017, (JMC), J. Miñano leg. [30SXG65]; Velez-Rubio, 840 m, wandering, 20 may 2008, (JMC), J. Miñano leg.; Vicar, 390 m, 1 ♂, wandering, 28 September 2009, (JMC), J. Miñano leg. [30SVG51]; ***Granada province***: Ceneveja la Vega, 750 m, 1 ♂, wandering, 02 July 2006 (JMC), J. Miñano leg. [30SVG51]; Benamaurel, 770 m, 1 ♂, wandering, 16 June 2012, (JMC), J. Miñano leg. [30SXH55]; Castilla-La Mancha: ***Albacete province*****:** Hellín, 580 m, 1 ♂, under a stone, 05 May 2003, (JMC), J. Miñano leg. [30SWG26]; Hellín, 500 m, 1 ♂, under stone, 20 September 2007, (JMC), J. Miñano leg. [30SXH16]; ***Ciudad Real province***: Ruidera, 800 m, 1 ♂, 27 October 1980, SHPC, M. A. Valentín leg [30SWJ11]; Alhambra, 860 m, 1 ♂, under stone, 20 September 2007, (JMC), J. Miñano leg. [30SWJ91]; Murcia Region: ***Murcia province***: Mazarrón, 200 m, 1 ♂, pitfall, 15 July to 30 November 1999, JKPC, Johan van Keer leg. [30SXG46]; Abanilla, 155 m, 1 ♂, pitfall, 01-30 October 2003, (JMC), J. Miñano leg. [30SXH87]; Alhama de Murcia, 380 m, 1 ♂, pitfall, 02-24 November 2005, (JMC), J. Miñano leg. [30SWG63]; Alhama de Murcia, 380 m, 1 ♂, wandering, 28 may 2005, (JMC), A. Millán leg. [30SXH42]; Cartagena, 150 m, 1 ♂, under stone, 28 August 2001, (JMC), J. Miñano leg. [30SXH62]; Cartagena, 175 m, 1 ♂, prey in web of *Steatoda paykuliana*, 01 may 2000, (JMC), J. Miñano & J. Martínez-Avilés leg. [30SXG86]; Cartagena, 5 m, 1 ♂, pitfall, 01-30 April 2008, (JMC), J. Miñano & BIOCIMA S.L. leg. [30SWF76]; Cartagena, 80 m, 1 ♂ + 1 ♀, manual collection, 02 June 1999, (JMC), J. Miñano & J. Martínez-Avilés leg. [30SXG86]; Cartagena, 80 m, 1 ♂, pitfall, 2 September 2006 to 23 September 2006, (JMC), J. Miñano & Environmental Volunteering CARM leg. [30SX G96]; Cieza, 370 m, 1 ♂, pitfall, 13 February to 20 March 2004, (JMC), J. Miñano leg. [30SXH94]; Fortuna, 190 m, 2 ♂, pitfall, 16 June to 15 July 2005, (JMC), J. Miñano leg. [30SXH62]; Jumilla, 640 m, 1 ♂, pitfall, 15 October to 15 November 2017, (JMC), J.L. Lencina leg. [30SWG86]; Jumilla, 690 m, 1 ♂, pitfall, 16 July to 02 August 2015, (JMC), J.L. Lencina leg. [30SYH13]; Jumilla, 840 m, 2 ♂, pitfall, 12 June to 28 June 2014, (JMC), J.L. Lencina leg. [30SXH61]; Lorca, 510 m, 1 ♀, manual collection, 23 June 2012, (JMC), J. Miñano leg. [30SXG17]; Lorca, 700 m, 1 ♂, under stone, 24 October 2006, (JMC), J. Miñano leg. [30SXH52]; Mazarrón, 170 m, 1 ♂, under plant *Stipa tenacissima*, 22 May 2003, (JMC), J. Miñano leg. [30SXG39]; Mazarrón, 190 m, 2 ♂, pitfall, 05 May to 20 June 2008, (JMC), J. Miñano leg. [30SXG45]; Mazarrón, 210 m, 1 ♂, under plant *Thymus hyemalis*, 20 February 2011, (JMC), J. Miñano leg. [30SWJ91]; Mazarrón, 70 m, 3 ♂, under plant, 26 February 2004, (JMC), J. Miñano leg. [30SXG45]; Murcia Municipality, 148 m, 2 ♂, pitfall, 01-31 June 2010, (JMC), V. Zapata & J. Miñano leg. [30SXH35]; Murcia Municipality, 170 m, 1 ♂, pool fall, 08 July 2004, (JMC), J. Miñano leg. [30SXH78]; Murcia Municipality, 175 m, 2 ♂, pitfall, 01-15 September 2006, (JMC), J. Miñano leg. [30SXG67]; Puerto Lumbreras, 425 m, 1 ♂, wandering, 14 July 2003, (JMC), J. Miñano leg. [30SXG39]; Ricote, 370 m, 1 ♂, pitfall, 01 June to 12 July 2010, (JMC), J. Miñano leg. [30SXG17]; San Javier, 125 m, 1 ♂, manual collection, 24 April 1995, (Lost), J. Miñano & J. Martínez-Avilés leg. [30SXG45]; Ulea, 155 m, 1 ♂, pitfall, 15 October to 11 November 2010, (JMC), J. Miñano leg. [30SXG35]; Yecla, 805 m, 2 ♂, pitfall, 25 April to 30 May 2016, (JMC), J.L. Lencina leg. [30SXH57]; Valencian Community: ***Alicante province:*** Agost, 360 m, 1 ♂, under a stone, 29 April 2006, (JMC), J. Miñano leg. [30SWG77]; Aspe, 280 m, pitfall, 03 May to 17 June 2013, (JMC), J. Miñano leg. [30SYH55]; Elche, 20 m, 1 ♂, under plant, 20 May 2008, (JMC), J. Miñano leg. [30SWG99]; Orihuela, 165 m, 1 ♂, wandering, 09 November 2008, (JMC), J. Miñano leg. [30SXH24]; Alicante

Municipality, 30 m, 1 ♂, manual collection, 20 February 2011, (JMC), J. Miñano leg. [30SXG47].

ADDITIONAL SPECIMENS ONLY RECORDED BY PHOTO: See Supporting Information (Figure S5.1-S5.12) for more details.

SPAIN: Andalucia: 1 ♂, 3 October 2009, Eva de Más (Figure S5.1); 5 ♂, 14 October 2010 (Figure S5.12), 10 October 2011 (Figure S5.13), 06 October 2012 (Figure S5.14) and 25 September 2014 (Figure S5.15), Francisco Rodríguez; 1 ♂, ***Granada province***: 4 October 2014, Fernando Molina Sánchez (Figure S5.16); 2 ♂, 30 September 2016 (Figure S5.17) and 3 October 2017 (Figure S5.18), Simon Oliver; Madrid Community: ***Madrid province***: 1 ♂, 6 October 2014, José Antonio Gómez (Figure S5.19); Murcia Region: ***Murcia province***: 1 ♂, 12 October 2007, José Luis Palacios, (Figure S5.110); Valencian Community: ***Alicante province***: 1 ♂, 2 October 2012, Stevie Smith (Figure S5.111); 1 ♂, 11 October 2005, Hugh J Griffiths (Figure S5.112).

ETYMOLOGY. The species is named after Dr Ben Collen (12 February 1978 – 19 May 2018), an internationally recognised conservation scientist (Jones & Purvis 2018) who loved Spain (the type locality of this species), where he taught numerous students and conducted multiple field courses. He supervised, supported and inspired the first author of this publication until his untimely death.

TAXONOMIC HISTORY. *L. annulipes* has, until this publication, only been recorded from Algeria, Tunisia, Egypt, and Israel. However, according to the "Araneae Spiders of Europe" website (Nentwig et al. 2018), this species is also recorded from southern Spain, quoting M. Řezáč (pers. comm.). This recorded occurrence has proven not to belong to *L. annulipes* but rather to the new species described here as *L. Colleni* sp. n.

SPECIES DESCRIPTION

Male. *Pattern and coloration.*-The most remarkable feature of *L. Colleni* sp. n. is the complete absence of a bright colouration pattern (Figure 5.**Error! Unknown switch argument.** and Figure 5.**Error! Unknown switch argument.**b,c), unlike the one found in the two other species of this genus, which always display bright red sections, both in the opisthosoma, as well as often in the prosoma (Figure 5.**Error! Unknown switch argument.**h, Figure 5.**Error! Unknown switch**

argument.g - h). *L. Colleni* sp. n. also has scattered white setae in the prosoma, which are often compact in the thoracic section (Figure 5.**Error! Unknown switch argument.**).

Figure 5.**Error! Unknown switch argument.** - General overview of the *habitus in vivum* of *Loureedia Colleni* sp. n. female (left) and male (right), both from Murcia Region.

Prosoma and legs tend to be whiter in individuals living in driest habitats with white soils where the only black regions in the male's prosoma are reduced to the ocular area and the chelicerae (Figure 5.**Error! Unknown switch argument.**). Individuals from less arid habitats have darker colouration patterns (black with brown shades).

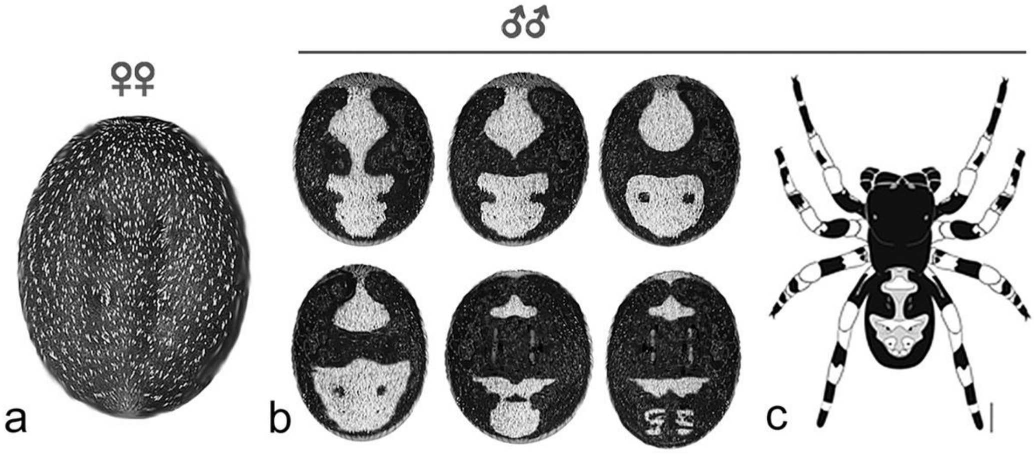

Figure 5.**Error! Unknown switch argument.** - Dorsal view of opisthosoma pattern in *Loureedia Colleni* sp. n.: [**a**] Females; [**b**] Males; [**c**] Schematic illustration of overall shape and colouration of males in *L. Colleni* sp. n., using colour code to represent the abdominal pattern variability. White areas represent regions where white colouration was always observed, and grey areas indicate regions where white colouration was at times

observed; dotted lines represent areas where the pattern has more often terminated (scale 1 mm).

The white rings found in this genus legs (probably the cause for *L. annulipes* etymology) are very broad in the *Loureedia Colleni* sp. n. and give the appearance of white legs interrupted by black rings (Figure 5.**Error! Unknown switch argument.**, Figure 5.**Error! Unknown switch argument.**c and Figure S5.1-S5.12). Although remarkably different in colour, the male's abdominal pattern in all *Loureedia* species shares some similarities, which we consider important to analyse as colouration is often lost in the preservation process. The anterior region is almost identical in all species to what concerns shape, and the region between the first two pairs of muscular insertions, although thicker in *L. annulipes* and *L. lucasi*, is also often present in *L. Colleni* sp. n. (Figure 5.**Error! Unknown switch argument.**c) The posterior region is where more distinctions can be noticed, as *L. Colleni* sp. n. pattern was always recorded as having white markings on the posterior muscular insertions (Figure 5.**Error! Unknown switch argument.**c, Figure 5.**Error! Unknown switch argument.**b), whereas in *L. annulipes* and *L. lucasi,* it is discoloured and merges with the lateral region of the black field (Figure 5.**Error! Unknown switch argument.**c, d, h; Figure 5.**Error! Unknown switch argument.**b, h).

Male genitalia. Although very similar to other male genitalia described for this genus, it presents several distinctive features, which we summarised (Figure 5.**Error! Unknown switch argument.**).

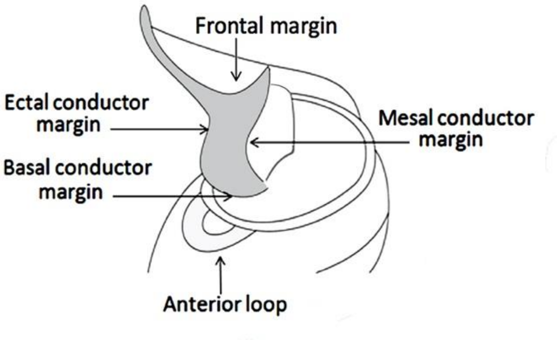

Figure 5.**Error! Unknown switch argument.** - Schematic illustration of the bulb pedipalp of *Loureedia Colleni* sp. n. ventral view with relevant morphological nomenclature.

Ventral view of the male pedipalp

Trait 1. The mesal conductor margin is very straight in *L. annulipes* (Figure 5.**Error! Unknown switch argument.**a), somehow sinuous in L. lucasi (Figure 5.**Error! Unknown switch argument.**b), and highly concave in *L. Colleni* sp. n. (Figure 5.**Error! Unknown switch argument.**, Figure 5.**Error! Unknown switch argument.**c), perhaps as a consequence of this feature, the angle formed by the mesal and basal conductor margins ends abruptly at a 90º angle in *L. annulipes*, producing an "L" shape (Figure 5.**Error! Unknown switch argument.**a), producing an inverted "c" shape in *L. lucasi* (Figure 5.**Error! Unknown switch argument.**a, Figure 5.**Error! Unknown switch argument.**b) and a "> " shaped corner in *L. Colleni* sp. n. (Figure 5.**Error! Unknown switch argument.**c).

Trait 2. The ectal margin of the conductor is only slightly curved inwards in the *L. Colleni* sp. n. (Figure 5.**Error! Unknown switch argument.**, Figure 5.**Error! Unknown switch argument.**c), in opposition to the highly concave ectal margin of *L. annulipes* (Figure 5.**Error! Unknown switch argument.**a) and the straight margin of *L. lucasi* (Figure 5.**Error! Unknown switch argument.**b).

Trait 3. The dorsal conductor tip is very similar between all species. Still, it is thicker at its base in *L. lucasi* and *L. Colleni* sp. n. and considerably straighter in *L. annulipes* and *L. Colleni* sp. n. The ventral conductor tip is also similar among all species, but it faces upwards at a slightly steeper angle in *L. annulipes*, making the margin that joins both tips closer to 90º.

Trait 4. From a ventral view, two sections of the sperm channel are visible through the tegulum of the conductor bulb. The posterior loop near the embolus is considerably smaller and thinner in *L. annulipes*. It has a much wider and ectal position in L. lucasi. In contrast, in *L. Colleni* sp. n. this section of the channel is both ectal and small, completing its anterior loop just at the edge of the conductor's basal margin.

Lateral view of the male pedipalp

Laterally, it becomes clearer that the dorsal conductor tip is not only longer in *Loureedia Colleni* sp. n. but also thinner, particularly at its tip (Figure 5.**Error! Unknown switch argument.**a, c), and its shape clearly distinguishes it from *L. lucasi* (Figure 5.**Error! Unknown switch argument.**a) and *L. annulipes* (Figure

5.**Error! Unknown switch argument.**a). In all three species, the tip of the ectal conductor tooth is raised higher than its base, but this is most notable in *L. Colleni* sp. n. even though the basal region of the dorsal margin is only slightly curved downwards (Figure 5.**Error! Unknown switch argument.**c) or even appear almost straight (Figure 5.**Error! Unknown switch argument.**a), while it is further curved downwards in *L. lucasi* (Figure 5.**Error! Unknown switch argument.**a) and strongly curved downwards *L. annulipes* (Figure 5.**Error! Unknown switch argument.**a).

The ventral margin of the ectal conductor tooth in *L. Colleni* sp. n. is highly convex throughout its entire length (Figure 5.**Error! Unknown switch argument.**c), while in *L. annulipes* it is only slightly convex (Figure 5.**Error! Unknown switch argument.**a) and is almost straight in *L. lucasi* (Figure 5.**Error! Unknown switch argument.**a).

In *L. Colleni* sp. n. mesal conductor tooth appears more exposed from the ectal one (Figure 5.**Error! Unknown switch argument.**c) because of its lesser torsion, which enables its tip to be more easily seen, while in *L. lucasi* and *L. annulipes,* a higher rotation towards its ventral side is required to expose it (Figure 5.**Error! Unknown switch argument.**a and Figure 5.**Error! Unknown switch argument.**a), which becomes clearer from a frontal view.

From a frontal angle, the mesal conductor tooth of *L. colleni* has lesser torsion, which is perceived as more open, wider, or as a less coiled spiral (Figure 5.**Error! Unknown switch argument.**c). In *L. lucasi* this torsion is greater and the tooth reaches further into the center of the structure (Figure 5.**Error! Unknown switch argument.**b), whereas in *L. annulipes* it has the highest torsion and is coiled even further (Miller et al. 2012, Fig. 63F).

Microstructures of the male palp

The frontal margin of the conductor appears different under SEM observation. It is smooth in *L. annulipes* (Miller et al. 2012, Fig. 63F), but serrated in *L. lucasi* (Gál et al. 2016, Fig. 4D) and *L. Colleni* sp. n. (Figure 5.**Error! Unknown switch argument.**).

From an apical view of the male pedipalp it is clear that the mesal conductor is linked to a complex flame-shaped lamellated structured -FSLS- (Figure 5.**Error! Unknown switch argument.**), which appears to be bi-dimensional, but it is in fact elaborated and probably has got taxonomic value in *Loureedia* or other Eresidae genera.

FSLS is a well-developed sheet in *Loureedia Colleni* sp. n., while is a small keel in *L. annulipes* (Miller et al. 2012, Fig. 63F) and has a row of flame shaped structures in *L. lucasi* (Gál et al. 2016, Fig. 4A, D) which are brush-like in appearance.

These micromorphological differences might be related to different reproductive strategies: 1) intraspecific recognition within a key-lock strategy, 2) to stimulate specific regions inside the female, 3) scraping internal structures within the female, to remove semen of competing males, for example (Eberhard 1996). Further research is needed to understand both the function of the FSLS and the sperm competition strategies in *L. Colleni* sp. n., as we have found mating plugs (Figure 5.**Error! Unknown switch argument.**b) and phenology data, as well as some empirical observations (Figure 5.1g) suggest that mate-guarding might occur.

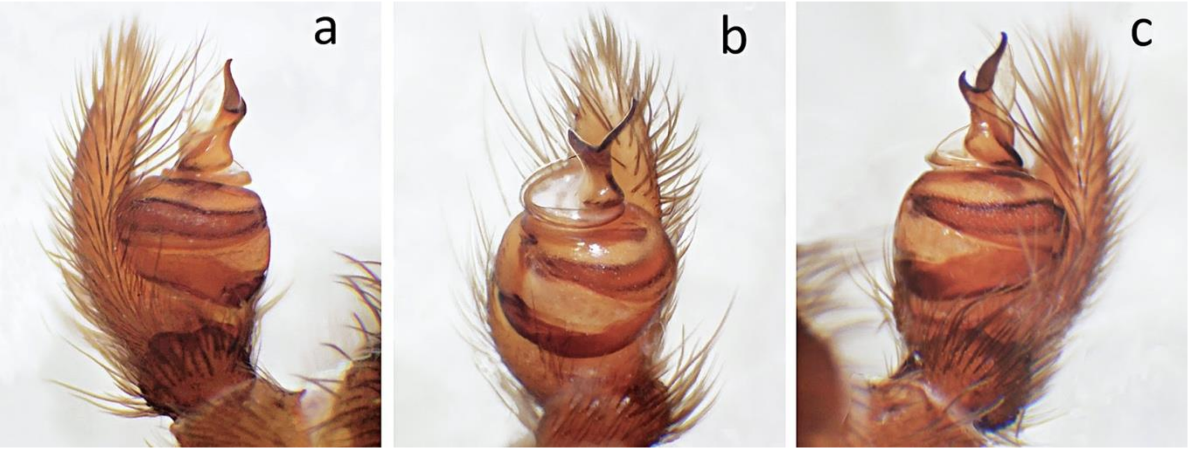

Figure 5.**Error! Unknown switch argument.** - Left male pedipalp of *Loureedia Colleni* sp. n.: [**a**] lateral mesal view, [**b**] ventral view, [**c**] lateral ectal view (turned).

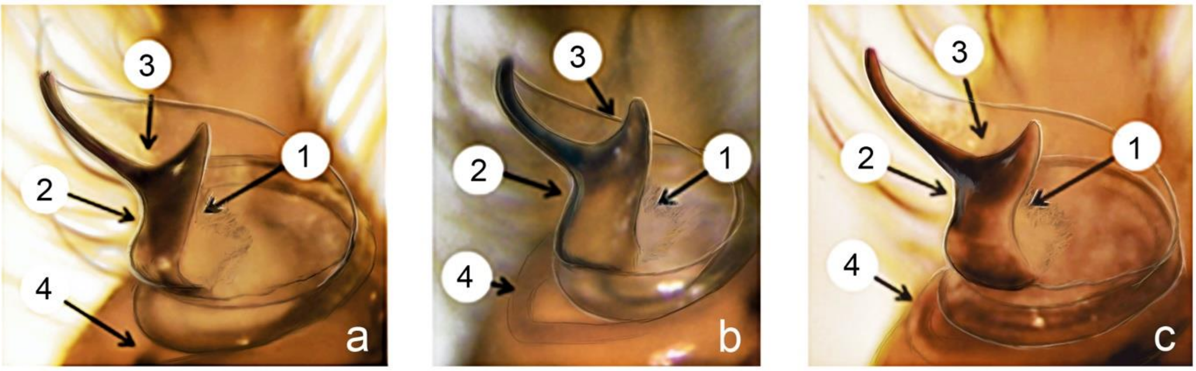

Figure 5.**Error! Unknown switch argument.** - Ventral view of male pedipalp (right) in the Loureedia genus: [a] L. annulipes (from Libya), [b] L. lucasi (type series), [c] L. Colleni sp. n. (holotype). Numbers 1-4 indicate the four traits considered most relevant to distinguish them (see results for more details).

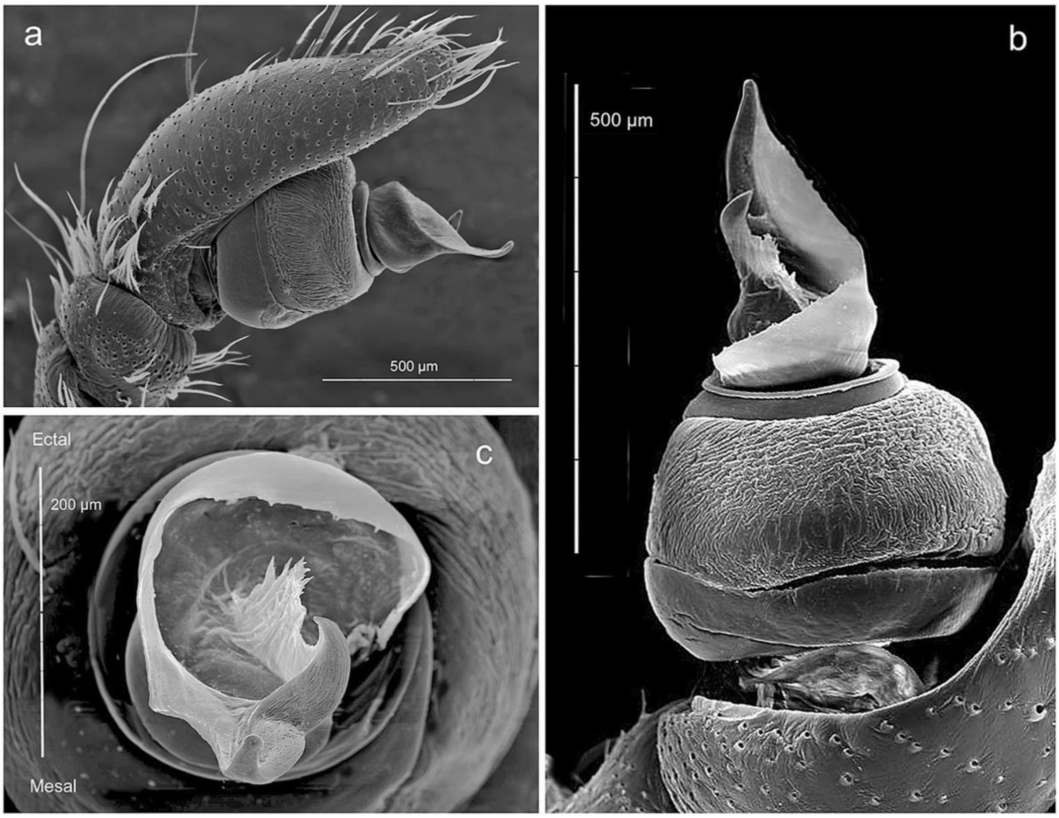

Figure 5.**Error! Unknown switch argument.** - Male pedipalp of *Loureedia Colleni* sp. n. under SEM: [**a**] lateral mesal view; [**b**] lateral ectal view; [**c**] apical or axial view.

Female. General greyish appearance due to the presence of white hairs between the black hairs in the background (Figure 5.**Error! Unknown switch argument.** and Figure 5.**Error! Unknown switch argument.**a), which can lead to misattributing to immature *Eresus* spp. Opisthosoma greyish background with white spots, larger in the anterior region, occasionally arranged in arcs in the posterior region. The carapace of prosoma often displays dorsal white marks that can be distributed in diffuse longitudinal stripes. Some females have a “mask” of yellowish hairs in the front ocular area and chelicerae surface, similar to some *Eresus* females (Figure 5.**Error! Unknown switch argument.**).

The legs are greyish in all their length or may present some white marks near the articulations of the segments. Some specimens can also present whitish marks that run longitudinally along most of the segments, especially in the legs I and II (Figure 5.**Error! Unknown switch argument.**).

Loureedia Colleni sp. n. females and males are often smaller than adult Eresus species found in the Iberian Peninsula, although considerable size variation can be observed in both genera. Measurements of the total body length are difficult to produce accurately for several reasons. The first one is that a considerable section of the prosoma is covered under the anterior part of the opisthosoma. The second one is that the opisthosoma volume can vary considerably depending on the abdominal gastric contents -making it an unreliable character. Furthermore, adult females can continue growing via new ecdysis after maturation, as moulting has been recorded in other Eresidae with completely developed female genitalia (Kraus & Kraus 1988).

Female genitalia. This structure is notoriously variable in the family, and the analysis present is not nearly as reliable as the one based on male genitalia. However, from the material currently available, the anterior lobe in *L. Colleni* sp. n. (Figure 5.**Error! Unknown switch argument.**c) can be much smaller than *L. annulipes* (Miller et al. 2012) both in height as in width, which in turn can be smaller than the anterior lobe of *L. lucasi* (Figure 5.**Error! Unknown switch argument.**e, f).

The size of the anterior lobe often positions the spermathecal heads much closer to each other in the *L. Colleni* sp. n. than in any other species and presents a very distinctive, almost lanceolate shape (Figure 5.**Error! Unknown switch argument.**b). In contrast, they are spherical in *L. annulipes* (Miller et al. 2012), and in *L. lucasi* (Figure 5.**Error! Unknown switch argument.**f) but much closer in the later, while almost pointing downwards.

The epigyne bar is always very visible both ventrally and dorsally (Miller et al. 2012) (Figure 5.**Error! Unknown switch argument.**e, f, Figure 5.**Error! Unknown switch argument.**c, d), dorsally it is almost straight in *L. annulipes* (Miller et al. 2012), concave in *L. Colleni* sp. n. (Figure 5.**Error! Unknown switch argument.**c) and obtuse in *L. lucasi* (Figure 5.**Error! Unknown switch argument.**e). The spermatheca is quite homogenous in width both in *L. annulipes* (Figure 5.**Error! Unknown switch argument.**d) and *L. lucasi* (Figure 5.**Error! Unknown switch argument.**f), but it becomes narrower at the base in *L. Colleni* sp. n. (Figure 5.**Error! Unknown switch argument.**d).

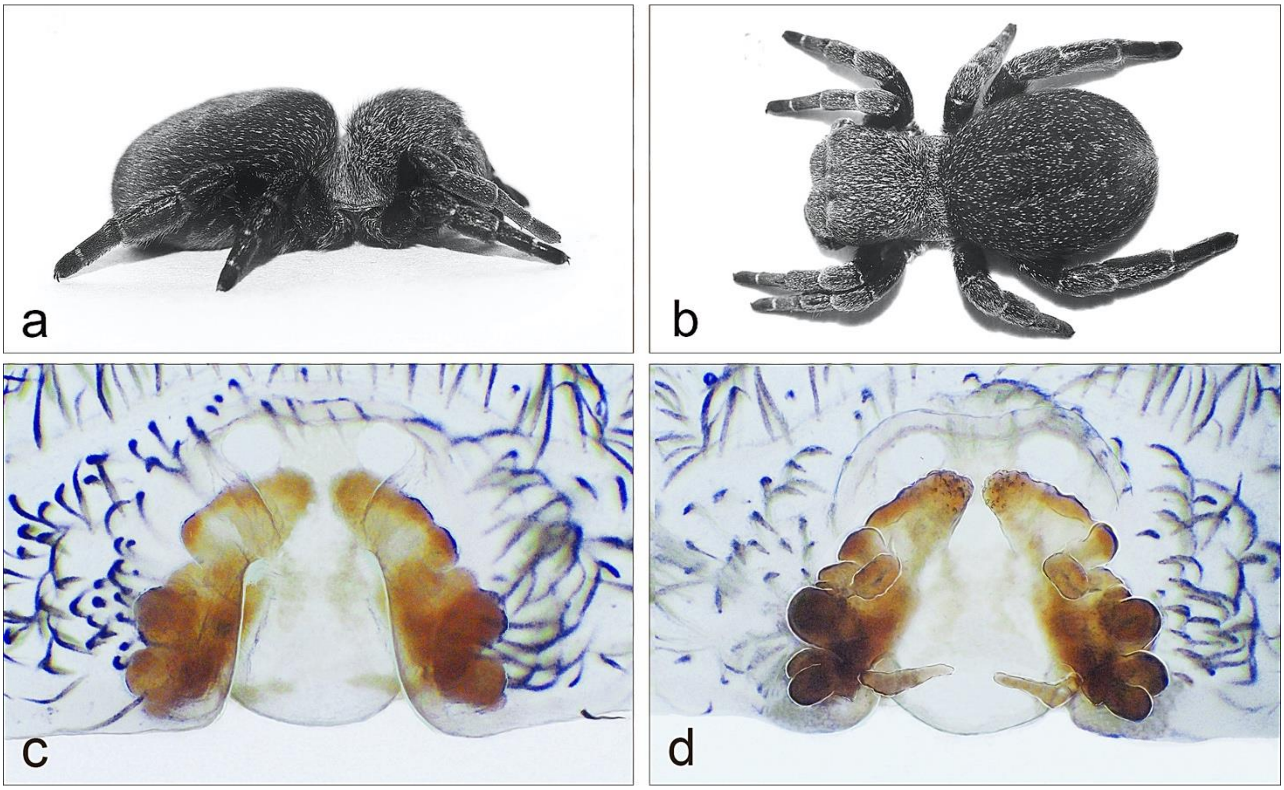

Figure 5.**Error! Unknown switch argument.** - Female *Loureedia Colleni* sp. n. (paratype). *Habitus in vivum*: [**a**] Lateral view, [**b**] dorsal view; Genitalias: [**c**] ventral view; [**d**] dorsal view.

HABITAT. This species is adapted to semiarid environments, with a preference for steppe habitats with good solar exposure and low density of scattered vegetation, such as shrublands or low shrubs with grasslands. In Mediterranean shrubland (with *Thymus* sp., *Rosmarinus* sp., *Anthyllis* sp., *Stipa* sp., *Lygeum* sp., among other species) and very open woodlands of *Quercus* sp. (in its northern range). It also appears in degraded areas such as re-wild farmlands and grasslands and in marginal areas of crops. Regarding soil type, *L. Colleni* sp. n. occurs in limestone, sandy, loamy and clay soils in both flat areas and hillsides (Figure 5.**Error! Unknown switch argument.**). Habitats with a mean annual temperature below 14ºC appear to be a limiting factor for this species. It is much more frequent in the lowest altitude levels and warm areas (15-18ºC annual mean temperature) than in more temperate zones occupied by Mediterranean forests (*Quercus* sp. and *Pinus* sp.). It occurs predominantly in areas with a high aridity index (average yearly precipitation below 350 mm coupled with strong potential evapotranspiration, four times higher than accumulated rainfall).

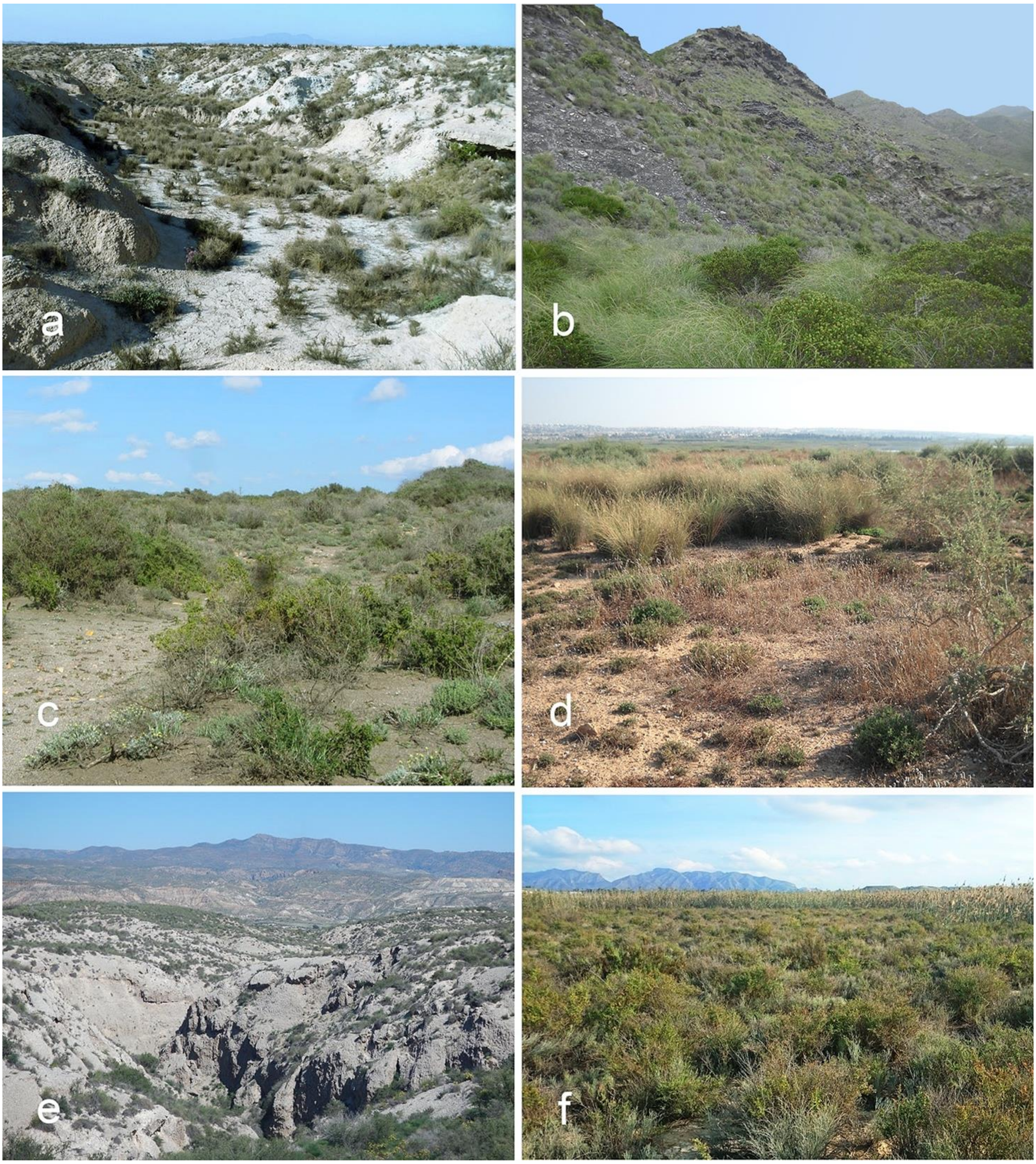

Figure 5.**Error! Unknown switch argument.** - Different habitats where *Loureedia Colleni* sp. n. has been collected: [**a**] Grassland of *Stipa tenacissima* with erosive clay loams. [**b**] Mediterranean shrub lands on metamorphic substrate. [**c**] Shrubland in a coastal sand dune ecosystem with secondary vegetation and stable soil. [**d**] Grassland of Poaceae in sandy soil. [**e**] Arid steppe in gypsiferous soils. [**f**] Steppe halophyte on margins of salt marsh wetlands.

DISTRIBUTION. The species proved to have a considerably large range, from sea level up to 800 m, occurring in Spain's southeastern and central regions (Figure 5.**Error! Unknown switch argument.**). Most of these natural habitats are threatened by urban development, extensive agriculture (mostly olive and almond tree growths), economic reforestation programs, and vast greenhouses (primarily in the south of Spain).

As most of these species populations are predicted to occur in some of Europe's hotter and driest regions (Hijmans et al. 2005), the species is likely to inhabit its critical or lethal thermal limits. As extreme weather events become more prevalent under climate change (Ezcurra 2006), the intensity and duration of thermal stress will likely increase, which might cause high mortality rates (Rezende et al. 2014) as this species is pushed outside its thermal tolerance.

Our models predict that at most 5% of *L. Colleni* sp. n. localities occur in the central and northern Iberian Peninsula (uncoloured areas in Figure 5.**Error! Unknown switch argument.**); populations in those areas are likely to inhabit poorly suited ecological niches with high fragmentation, which has likely been exacerbated by human activity over the last century and particularly over the last decade. The probable low dispersal ability of this species, as inferred from the low dispersal ability of other members of the same family (Řezáč et al. 2008), indicates that such small isolated populations are unlikely to support long-term viable colonies of the species, even as climate change causes these areas to become more thermally suitable for this species (Ezcurra 2006).

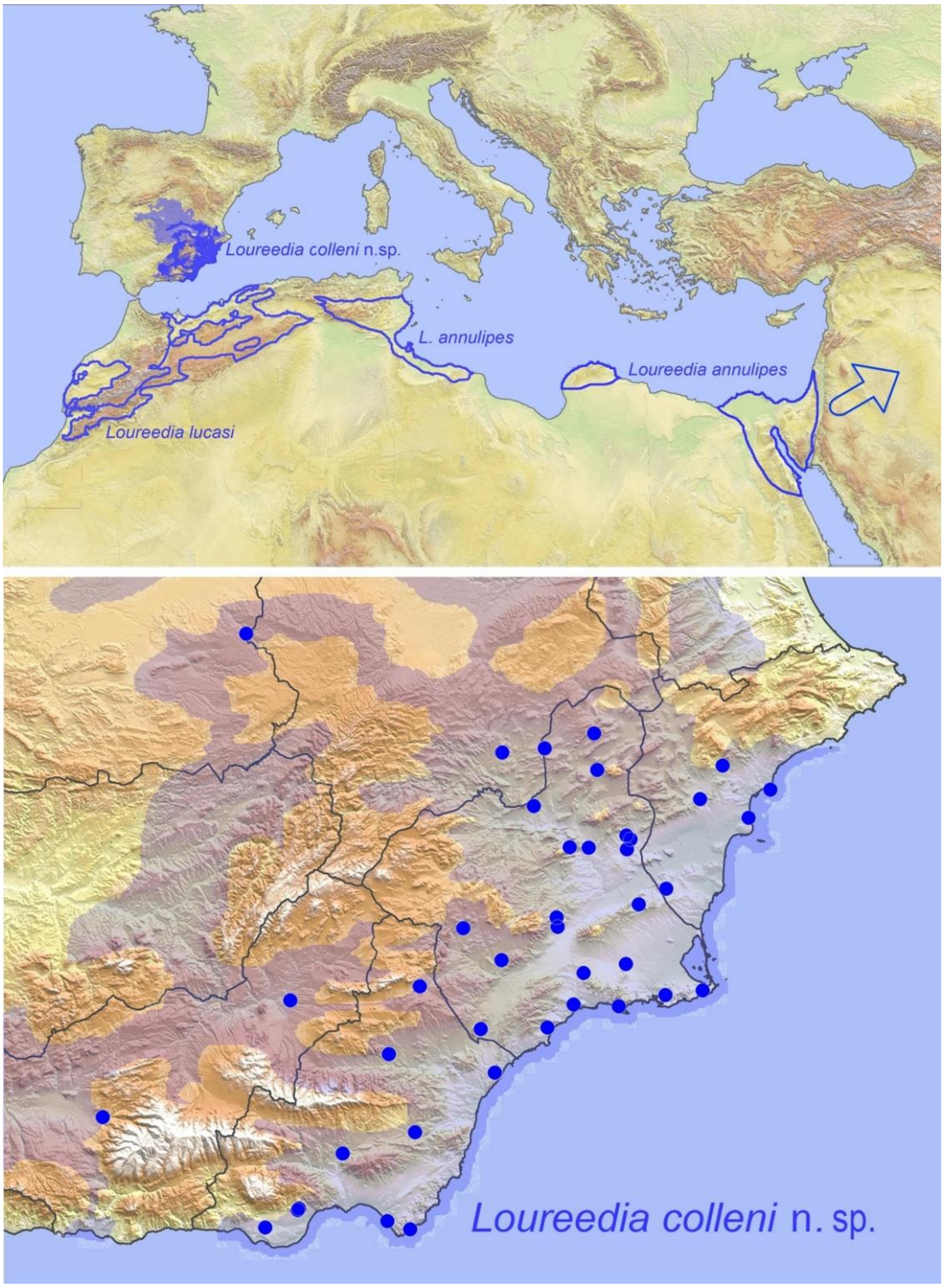

Figure 5.**Error! Unknown switch argument.** - Potential species distribution of *Loureedia Colleni* sp. n.: Mediterranean Basin using coloured polygons to represent the potential distribution (area where 90% of localities are expected to occur in dark blue, both tones of blue representing the area where 95% of localities are expected to occur in both tones of blue) and using empty polygons to represent the potential distribution of *L. annulipes* and *L. lucasi*, empty arrow indicates the direction of the Iranian record [Above]. Detail of the southeast of the Iberian Peninsula, potential species distribution of *L. Colleni* sp. n. illustrated as a darkened area (90% probability of occurrence), blue circles indicating known records of the species [Below].

BIOLOGY: Phenology: Most of our records and observations of *L. Colleni* sp. n. males occurred mostly in two periods, during Spring and Autumn (Figure S5.1-5.12). Males of the species appear to search for females throughout most of the year, except for January (coldest month) and August (driest month), especially in the hotter and most arid areas of its distribution (with mean annual temperatures above 16º C).

Nest: *Loureedia* species build burrows about 10 cm deep in several types of soil (Figure 5.**Error! Unknown switch argument.**), where they remain most of their lives inside a refuge zone. Laterally, from the hole, they weave a funnel with a dense and insulating mesh of silk, which they use as a hunting zone (Figure 5.**Error! Unknown switch argument.**b). This funnel is covered by a white-silk sheet raised from the ground with tensioning threads and where individuals add some vegetal material or clay debris, which they use as a concealing zone (Figure 5.12a) and under which they store most of the prey remains, as a storage zone (Figure 5.**Error! Unknown switch argument.**a). Nests can usually be found in open areas but are more often found under shrubs, occasionally built partially or completely under a rock (Figure 5.13a). Nests might be found without a vertical refuge area (as found elsewhere) but will rather have an irregular horizontal burrow.

Figure 5.**Error! Unknown switch argument.** - Nests of *Loureedia Colleni* sp. n.: Undisturbed active nest in soil, and same nest after raising the white-silk sheet, exposing the structure of the funnel and burrow leading to the refuge zone.

<u>Diet:</u> Prey remains in nests mainly include Tenebrionidae, and in nests close to wetlands, Isopoda.

Some identified prey are *Scaurus rugulosus*, *Opatrum* (*Colpophorus*) *baeticum* ssp. *almeriense* and *Tentyria* sp. (Figure 5.**Error! Unknown switch argument.**a).

<u>Reproductive traits:</u> Females of *Loureedia Colleni* sp. n. have been found with mating plugs (Figure 5.**Error! Unknown switch argument.**b), and there is evidence to suggest that the *Loureedia* genus can practice sexual cannibalism (Figure 5.**Error! Unknown switch argument.**g). In other spider species with low paternal investment but with frequent sexual cannibalism, non-promiscuous mating was recorded, and evidence suggests that it predisposes males to become competitive and selective towards females (Andrade & Kasumovic 2005). Similarly, if *Loureedia* mating plugs prove effective at preventing females from being inseminated by multiple males, and the single male who does get to mate is killed by the female or dies while guarding her (terminal investment), this would entail that *Loureedia* spiders would be physically unable to practice promiscuous mating and would suggest that in these monogamous species, the males are also likely to become competitive and selective towards females.

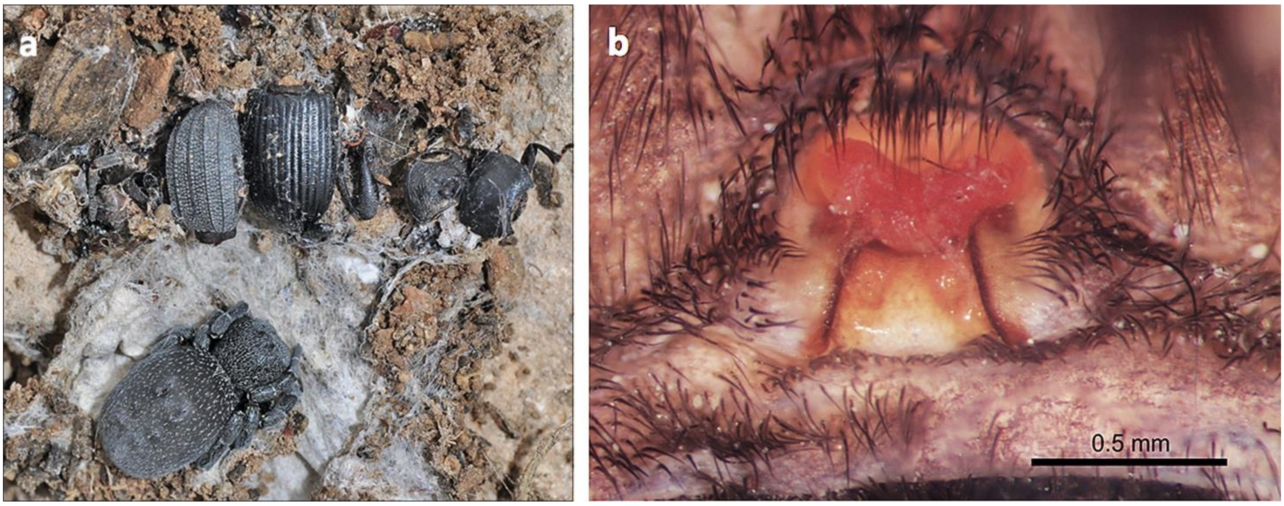

Figure 5.**Error! Unknown switch argument.** - Adult female of *Loureedia Colleni* sp. n. (paratype), in her nest with prey remains [**a**]. Female with mating plug (second female paratype) [**b**].

5.2.4 Discussion

The analysis of a broader set of species of *Loureedia* spiders allowed us to reanalyse the traits that were originally set to define the genus based solely on *Loureedia annulipes* (Miller et al. 2012).

The bifid conductor process, which was pointed out as a unique feature of this genus (Miller et al. 2012), is present both in *L. Colleni* sp. n. and in *L. lucasi* and should remain the most distinctive feature of the group, although bifurcation also occurs in the pedipalps of *Stegodyphus dumicola*, *S. tentoriicola*, and *Paradonea striatipes*, it does not occur to the same extent, as these aforementioned species only have terminally bifid processes, whereas, in all *Loureedia* species, the conductor tip is entirely composed of two bifurcating teeth, a ventral tooth and a dorsal one which bears a basal lamella (Figure 5.**Error! Unknown switch argument.**), which is similar to the structural complexity of the pedipalp in other Eresidae, but highly distinctive in its shape.

Posterior medium eyes (PME) are clearly larger than the anterior medium eyes (AME) in all *Loureedia* species (AME/PME ca. 0.5) as well as the lack of prominent tubercles bearing the ALE. However, these are not unique features to this genus they do allow it to be easily differentiated from *Dorceus* and *Dresserus*, respectively (Miller et al. 2012).

Once that previous analyses on the Loureedia genus had reduced access to females (Miller, pers. comm.) or no access to female specimens at all (Gál et al. 2016). Analysis of distinctive genus traits in females has been lacking. Once we were able to analyse females of all the recognised species, we observed that the epigynum morphology of this genus is also very distinct from other eresids, with its unique anterior depression and the compact configuration of the reproductive duct system.

The striking black-and-white colouration of L. Colleni sp. n. males is so distinct from other Loureedia species that it allows for photographic records to be reliably identified to the species level (supporting information Figure S5.1-S5.12). However, despite this remarkable colour difference, all *Loureedia* males analysed in this publication share strong morphological similarities and a common pattern design (Figure 5.**Error! Unknown switch argument.**d, h, Figure 5.**Error! Unknown switch argument.**b, g, h, Figure 5.**Error! Unknown switch argument.**[bellow], Figure S5.1-S5.12).

Contrary to the clearly distinct *Loureedia* males, the females have a simple design and can be easily mistaken with *Eresus* females, with which they can share their habitat. Therefore, photographical diagnosis of females is not recommended as an accurate record in this group. Nonetheless, female sightings are rare, as only males disperse to find a partner, and the more reclusive and cryptic *Loureedia* females do not present strong colouration in the anterior cephalic regions, whereas *Eresus* females often do.

The cephalic region shape, described as potentially distinctive in the original genus description (Miller et al. 2012), is not present in all species and, as a genus character, must now be redefined to encompass all three species. We observed that it is wider than long in *L. annulipes* and longer than wide in *L. Colleni* sp. n., rendering this character unreliable for this genus distinction with *Dresserus* and *Paradonea*.

As mentioned earlier, females can be challenging to identify or distinguish by photos alone, but males of L. Colleni sp. n. are very distinct from any other Iberian eresids, and photographic records have and should continue to be, used to monitor this species' distribution, phenology or even population density. We consider this to be a valuable trait, as it places *L. Colleni* sp. n. as a potential model species to develop citizen science and non-invasive species monitoring.

It appears difficult to distinguish between *L. annulipes* and *L. lucasi* by photographic record alone. Still, the genus pattern design is surely distinctive, and we hope this publication will drive the research of this genus further, to the point where we can better understand the distribution of these species across the genus range.

Despite the striking differences between the black and white males of *L. Colleni* sp. n. and the brightly red colouration of the *L. annulipes* and *L. lucasi* males, both colouration types appear to be aposematic in nature, as *L. Colleni* sp. n. is quite a noticeable species against the mostly brown, light green background of the Southern Spanish areas where it occurs. As we know very little about *L. Colleni* sp. n. ecology, it is difficult to know how this species is perceived by its predators and if its colouration could be considered cryptic in some way (when considering the UV spectrum, for example). This is beyond the scope of this work but should be the focus of future research.

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5.4 Supporting Information

Figure S5.1 – S5.6 Edited photos from the original photographic records of *Loureedia Colleni* sp. n. [S5.1] by Eva de Más in 2009; [S5.2] by Francisco Rodríguez in 2010; [S5.3] by Francisco Rodríguez in 2011; [S5.4] by Francisco Rodríguez in 2012; [S5.5] by Francisco Rodríguez in 2014; [S5.6] by Fernando Molina Sánchez in 2014.

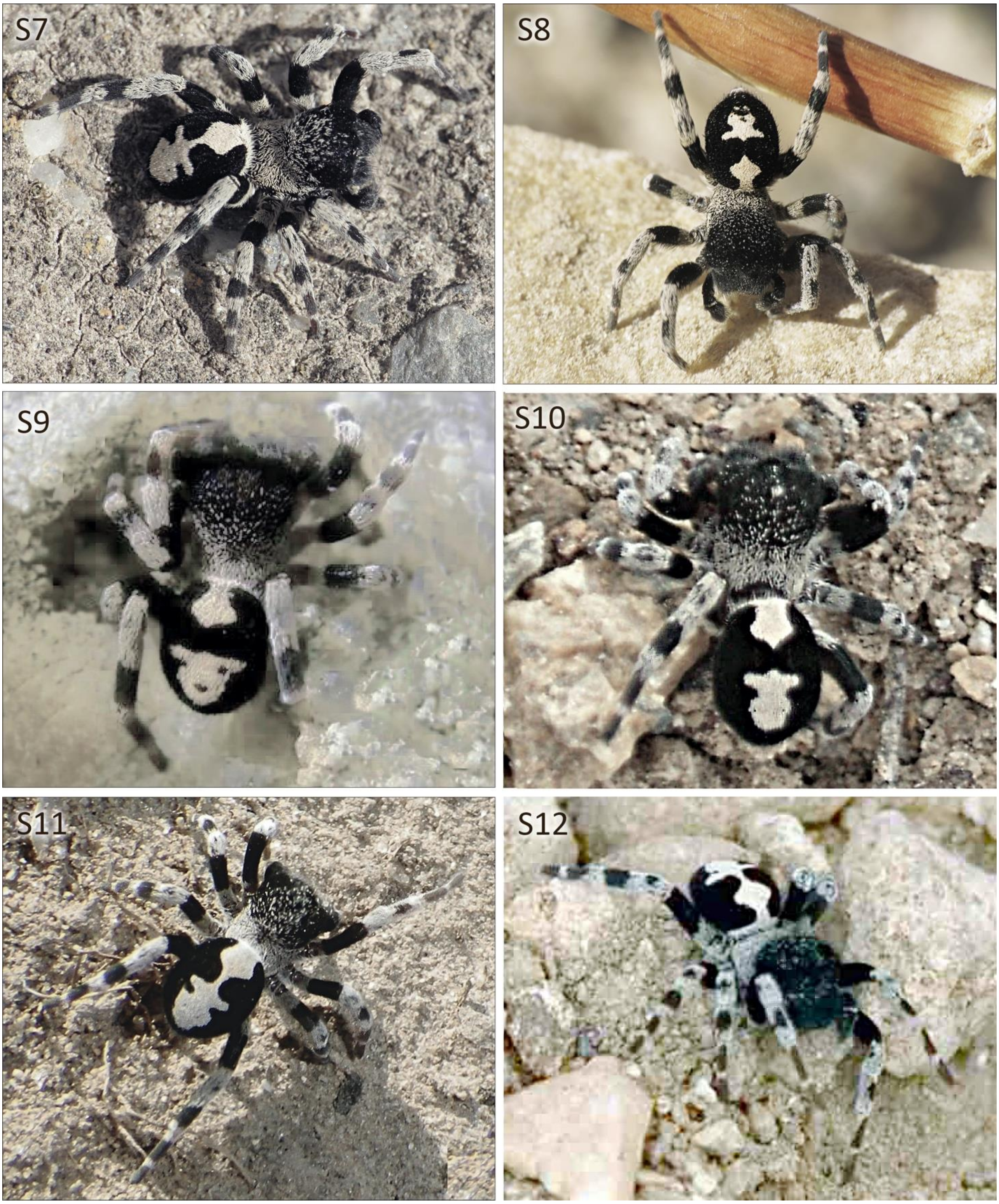

Figure S5.7 – 12 Edited photos from the original photographic records of *Loureedia Colleni* sp. n. [S5.7] by Simon Oliver in 2016; [S5.8] by Simon Oliver in 2017; [S5.9] by José Antonio Gómez in 2014; [S5.10] by José Luis Palacios in 2007; [S5.11] by Stevie Smith in 2012; [S5.12] by Hugh J Griffiths in 2005.

Chapter 6

Synthesis

6.1 Concerns and opportunities

The concerns expressed by Marianne North in the 1800s, “of man, the civiliser, wasting [natural] treasures” are sadly still justified today, as despite many conservation successes we are still in a biodiversity crisis and failing to meet internationally set biodiversity targets to stop it (CBD 2020a, b). There is an ongoing discussion about what type of biodiversity indicators we should be using (Farooq et al. 2020; Hughes et al. 2020; Soto-Navarro et al. 2020; Williams et al. 2020), including what targets are feasible to achieve and how many indicators may be needed to detect progress towards current biodiversity goals, under fears of “index bloating” (the proliferation of indicators). I found efforts towards validating and expanding the biodiversity indicators we already have to be less prevalent in the literature and therefore set out in this thesis to explore ways to expand the use of the Red List Index (RLI), one of the most used biodiversity indicators to date.

The cumulative results presented in this thesis are an interconnected approach, where I tested how small a representative sample of a group’s RLI (sRLI) trend can be and how the data gathered towards that sample might also contribute towards detecting the proportion of threatened species in the group. This provides a simple but powerful way to convey the ongoing biodiversity crisis to policymakers and the general public.

Remarkable efforts have led to the extinction risk assessment of 128,918 taxa; this number, however, represents approximately 5% ’ of the world's 1.7 million recorded (IUCN 2020), out of a conservative estimate of 8.7 million extant species (Mora et al. 2011), placing the number of assessed species to less than 1% of Earth’s biodiversity (chapter 1). Furthermore, as anthropogenic material surpasses that biomass (Elhacham et al. 2020) and even though conservation has been prioritising the protection of larger species (e.g. panda), we do not yet have extinction risk assessments for most of the planet's biomass (Bar-On et al. 2018).

The sRLI is a promising approach towards reducing this knowledge gap. It was established as an approach to better estimate the extinction risk of lesser-studied groups and has provided excellent baseline estimates of the extinction risk of many groups. This included plants(Brummitt et al. 2015b), fungi (Minter 2011), and terrestrial (Böhm et al. 2013) as well as freshwater animals (Böhm et al. 2020). However, it has proven hard to implement twice to understand how that extinction risk is changing. Therefore, we still do not know if extinction risk is increasing or decreasing in these groups.

One of the obstacles to the implementation of the sRLI is the size of the recommended sample (900 non DD species). I showed that dramatically lowering the sample size to 200 species could provide sufficient information to achieve the original aim of the sRLI in all available non avian groups (Henriques et al. 2020). If this sample size were adopted, it would considerably accelerate our understanding of species extinction risk, in particular towards groups we know to be under serious threats but are still severely understudied (e.g. scorpions) or groups we have not measured in over a decade (e.g. amphibians), and where this sample size could inform if extinction risk has continued to decline or was halted. Meanwhile, I also found groups that have implemented the previously proposed sample size (900 sp.) should continue to reassess that sample whenever possible, as that would allow us to detect changes in slope (Henriques et al. 2020) towards discovering if extinction risk is accelerating or slowing down.

A sample, by definition, is not set to give us an exact answer but rather an approximation of the truth. Although some of the current literature has assumed a binary interpretation of results as either "wrong" or "right" (e.g. Ioannidis 2005), we find this to be a reductionist view of the natural world and statistical analysis of samples in particular. In our analysis, this true/false dichotomy did not apply, but instead, we are presented with a likelihood of detecting the correct RLI trend or presented with a distance to the actual proportion of threatened species value, where we are either reasonably close to, or far away from the "truth". Those are the trade-offs I presented (chapters 2 and 3); there is no absolute sample size that can give us the right answer; all one can do is consider what we aim to detect in order to accurately present how confident we are in our results, and how accurate those are likely to be. How to do that, in this particular context, is the set of tools this thesis presents to the community.

The sRLI has been applied to various taxonomic groups, which are currently used to inform widely reference estimates of how many threatened species there are globally (IPBES 2019). This estimate is based on the proportion of threatened species, a metric that the sampled approach was never tested to detect. Therefore, I explored how accurate current estimates might be in sampled groups and found that the error they might contain was often dwarfed by the number of data-deficient species (chapter 3).

The impacts our results could have on the global estimate of 1 million threatened species is sizable, potentially adding a quarter of a million species even under a conservative margin of error (i.e. 0.05). Because insects represent half of this estimate (Purvis et al. 2019), we desperately need to sample further insect orders globally if we aim to increase its accuracy. The assessment of insect groups takes on increased urgency if one considers the growing amount of evidence detecting worrying declines in insects. A crucial conservation issue that current roadmaps recognise can be best averted if extinction risk data are made available (Harvey et al. 2020).

Promising progress has also been made towards this goal, such as the EntoGEM systematic mapping project (Grames et al. 2020; https://entogem.github.io), a community wide that aims to assimilate scientific literature and available data sets of insect populations as well as their diversity trends. These types of projects are crucial efforts if we want to detect and monitor global biodiversity trends, as currently available groups, such as vertebrates, have repeatedly proven to be poor indicators of biodiversity loss in a variety of ecosystems (Estrada & Rodríguez-Estrella 2016; Ruaro et al. 2016; Siddig et al. 2016; Rodríguez-Estrella et al. 2019). Whereas arthropod groups, such as spiders and insects, have been reliably used to indicate environmental disturbance in freshwater and terrestrial ecosystems for decades (Maelfait & Hendrickx 1997; McGeogh 1998; Rainio & Niemelä 2003; Avgın & Luff 2010).

Arthropods, in particular (like sea spiders or crustaceans) and invertebrates overall (such as molluscs) are also crucial parts of marine ecosystems, which currently face increasing threats, such as deep sea mining (Dunn et al. 2018). This is a gap that increasing our understanding of insects will not fill. Therefore, I would recommend aiming future conservation priorities to focus not solely on insects (which mostly occur in terrestrial ecosystems) but on arthropods more generally, so we risk misestimating the decline of species that inhabit three-quarters of our planet.

From the onset, I was also aware that accelerating the sampled approach to the RLI (chapter 2) and broadening its scope to detect the percentage of threatened species (chapter 3) could be useful. However, the obstacles to the sampled approach would only truly be understood when trying to implement it in a poorly studied megadiverse group (chapter 4). Pursuing this path gave me the opportunity to explore the main challenges, which allowed me to gain a deeper appreciation for the impactfulness of data deficiency.

The conservation field is not the first to face such data gaps. Quantifying the bias introduced by nonresponse to questionnaires and other survey methods is, statistically speaking, similar to the inability to know what the extinction risk of a DD species is. Under the sampled approach to the Red List Index (sRLI), a species that is assessed as DD becomes, in effect, excluded from the sample as it does not contribute to the RLI value (Baillie et al. 2008). This exclusion is, in essence, not different from a human participant leaving a research trial, where it is known that there may be systematic differences between those who leave the study and those who continue. Still, the bias that can be introduced into the study is unknown. This type of missing information is known as nonignorable nonresponse, more often called missing not at random (MNAR), and it causes attrition bias (Ghanem et al. 2020; Taylor et al. 2020), relating to differential attrition between participants or species, under the sRLI framework.

There are several methods to deal with attrition bias, which include using the last observation or the baseline value and carrying it forward (e.g. Petroff 2017). However, this is not possible to implement under the sRLI framework as DD species extinction risk was never assessed successfully in the past. Another method to deal with this issue is multiple imputation (MI), which replaces missing values (i.e. species IUCN Red List categories) with imputed values (Murray 2018).

One approach to MI is the use of uncertainty intervals (Gamble & Hollis 2005). These, however, are not applicable to address DD under IUCN criteria because the DD uncertainty interval should always be between Least Concern and Critically Endangered or even Extinct (IUCN Standards and Petitions Committee 2019). A shorter interval should activate the precautionary principle, where the highest feasible threat category should be the one assigned (IUCN Standards and Petitions Committee 2019). Another approach to MI is the best-worst sensitivity analyses (e.g. in the best case scenario, all DD are Least Concern, or in the worst case scenario, all DD are Extinct), which was the one I found most useful and practical to implement (see chapter 4) and is currently the approach followed by the IUCN (IUCN 2020). Yet another approach to MI is reference-based multiple imputation, which uses imputed values based on another reference dataset, usually other treatment arms in trial studies (Leurent et al. 2020). This technique has long been used by IUCN by assuming that all DD species were as threatened as the reference dataset of non-DD species and was also used in this thesis. However, as I point out in chapter 4, several conservation studies have detected DD species to have higher threat levels than non-DD species.

Another commonly used approach to MI is the use of mixed models, which require large datasets (Jetz & Freckleton 2015) and include species-level trait-base data (Luiz et al. 2016). Although the relationship between most traits and extinction risk remains elusive (Chichorro et al. 2019), it is one of the most widely used approaches (Evans et al. 2018; González-del-Pliego et al. 2019; Walls & Dulvy 2020). However, such a data set is not yet available for many of the groups under the sRLI, including spiders. Therefore, it was not an approach we could use at this point, although advances towards building a trait database for spiders may be forthcoming (Lowe et al. 2020).

What I found in practice, when conducting an exploratory analysis of DD species in our sample (supplementary information S6.1), is that there is still a lot of potential to find further data that can add valuable information towards targeted surveys or the extinction risk assessment of species we preliminarily classified as DD. However, this will always remain an initial (desktop) assessment that needs a second (ground-truthed) assessment (Brummitt et al. 2015a). I learnt that the case study tackled in this thesis, spiders, should be seen as an opportunity not to find better statistical tools to infer the extinction risk of DD species but instead to acknowledge the importance of taxonomic research and target surveys (ground-truthing) if we truly want to understand the extinction risk of global biodiversity.

The points I have summarised, however important, do not impact the underlying fact that regardless of the data we are able to gather and the accuracy of biodiversity indicators, these can only confidently detect how well we are performing towards halting the current biodiversity crisis. In the end, it is down to all of us to be the change we can detect.

In 2020, amid a global pandemic, unlike the previous zoonotic outbreaks of HIV, Ebola, SARS, Bird Flu, and MERS, COVID-19 might finally become a turning point for a number of issues, including how urgent it is to stop the global biodiversity crisis (Lorentzen et al. 2020). Besides causing substantial declines in global species richness, habitat loss also influences the risk and emergence of infectious zoonotic diseases, like COVID-19, in humans (Myers et al. 2013; Gottdenker et al. 2014; Khetan 2020), as well as increasing zoonotic host diversity (Gibb et al. 2020).

If there was ever a time to drive policymakers into action, it would be now. Yet even in wealthy nations such as the UK, apparently generous pledges for an extra £40m investment into the government’s Green Recovery Challenge Fund (UK Government 2020) represent less than 8% of the amount pledged by the same government for the “Eat out to Help Out” scheme (that lasted less than a month). This scheme was meant to support small businesses but resulted in 8 to 17 per cent of new COVID-19 infection clusters (Fetzer 2020). Meanwhile, a few days earlier, a report revealed the same UK government continues to make crippling cuts to Natural England, responsible for protecting and improving England’s natural environment, whose programme expenditure has dropped by £30m since its formation (Prospect 2020).

The UK example is an interesting case study, as the disparity in investment sends a clear message on the perceived importance of different issues. The discrepancy between cuts and support, besides reflecting how current British policymakers are underfunding research and actions to halt the biodiversity crisis, also reveals a disconnect from the UK government's own data. The Green Recovery Challenge Fund was revealed to be insufficient by Environment Minister Rebecca Pow herself, who disclosed that previous pledges of the same value were almost seven times oversubscribed (Harrabin 2020).

This sequence of events leads me to wonder for how long Marianne North's 1800 quote will remain true that it breaks “one's heart to think of man, the civiliser, wasting [natural] treasures...” (North 2011).

6.2 Summary of findings

The main objective of this thesis was to understand how extinction risk assessments could be better made of lesser-known and megadiverse groups such as Araneae. To understand this, I investigated how the sampled approach to the Red List index could be optimised and updated in light of recent assessment of all species in several taxonomic groups and current policy targets. Implementing this protocol in practice on a megadiverse group gave me a deeper understanding of the main constraints of using this protocol and how to overcome them.

In chapter 1, I introduced the historical and political context which led to current international biodiversity targets, the biodiversity indicators that have been proposed to detect our performance towards achieving them, and the metrics they are based on. I focus in particular on one of the most widely used biodiversity indicators, the Red List Index (RLI) and the metric it is based on, the IUCN Red List of Threatened Species. I showed how, despite the impressive amount of species currently included in the IUCN Red List, it represents a non-random sample of global biodiversity, which limits the questions that it can answer, such as the impacts of specific threats (e.g. climate change, invasive species, trade) on global biodiversity.

In Chapter 2, I revised the sampled approach to the RLI (sRLI), a promising way to overcome current knowledge limitations that are aimed at detecting a group's RLI trend direction by assessing a random sample of its species. I show how, by analysing a wider dataset than the one available at the inception of the sRLI, under current policy targets and accounting for the time period between the assessments that form a trend, a considerably smaller sample of 200 sp. was able to detect the RLI trends of all available non-avian groups (birds required a 400 sp. sample). I also showed that the currently proposed sample size of 900 sp. was suitable to correctly detect changes in slope (i.e. it allows us to detect when a group is declining faster or its decline is slowing down). I found that only very shallow slope changes require the entire group to be assessed to detect these (Henriques et al. 2020).

In chapter 3, I acknowledge that time is of the essence under the current biodiversity crisis and that despite the importance of measuring trend direction and slope changes, there is an urge for biodiversity indicators to inform policy targets now. I show that random samples of extinction risk assessments that can form the baseline for an RLI trend detection can also inform the group's proportion of threatened species. Specifically, I found that the currently recommended sample sizes of 900 sp. detected the proportion of threatened species in target taxonomic groups within a 2.5% error of the known value, with 95% confidence, while under the same confidence level, much smaller samples of 300 sp. where sufficient to detect the known proportion within a 5% error. From my analysis, I concluded that how close one wants to be to the true value of the proportion of threatened species should impact the choice of sample size one chooses to assess. I also show that while samples of 200 sp. may be considerably more feasible to implement, they can have a 6% error with 90% confidence. My results revealed that while the error a sample can have is important to consider in the choice of sample size, in currently sampled groups, this can be surpassed by the uncertainty caused by Data Deficient (DD) species. The proportion of threatened species is already used to communicate biodiversity decline in multiple policy settings. In light of our results, we propose that it should be considered as a biodiversity indicator used towards detecting the success of international biodiversity targets.

In chapter 4, I implement the lessons from chapters 2 and 3 on a case study that can benefit the most from a sRLI, a poorly studied megadiverse group. With my collaborators, I assessed the extinction risk of a random 200 sp. sample of global spiders for the first time. I found that most (70.5%) of the species selected warranted DD status, a main obstacle to assessing the extinction risk of this group. I propose a shift in how DD has been addressed under the sRLI framework towards targeted efforts that could pragmatically address their data deficiency, where we used the spatial distribution of DD species in our sample to highlight where targeted surveys would be most efficient. However, we also note that one of the main causes of DD is still lack of taxonomic research.

In chapter 5, I tackle what we found to be one of the main causes of DD: taxonomy. I summarise how taxonomy impacts all stages of the sRLI, from species selection to their extinction risk assessment and reassessment, set to take place a decade later under current IUCN guidelines. I give clear examples to show that the selection of species included in a sample, replaced or excluded due to updated taxonomic research, can cause attrition bias. I propose ways to standardise current approaches to this issue under the sRLI. I also demonstrate the importance of including newly described species into the sRLI, in order to reduce the risk of potentially excluding some of the most threatened species in a group and propose a clear approach to do so when reassessing the group extinction risk towards estimating its RLI trend. I show that it is not only DD species that are impacted by taxonomy, but what is colloquially called “lumping” and “splitting” (several species being synonymised under the same name or a taxonomic entity containing multiple species) can also have a considerable impact on species extinction risk assessment. I present *Eresus* and the closely related *Loureedia* genus as a case study that illustrates many of these taxonomic challenges and their impact on IUCN Red Listing and take the first steps towards addressing them.

6.3. The path ahead

We are at a crossroads where we can still reverse the current biodiversity trends (Leclère et al. 2020) but continue to fail to meet internationally agreed biodiversity targets (United Nations 2020). As Ellman Soulé wrote at the birth of our field “although crisis oriented, conservation biology is concerned with the long-term viability of whole systems” (Soulé 1985). This presents a dilemma where our focus should be on long-term outcomes, but a number of factors compel us to invest energy and other resources in snapshot indicators.

In this thesis, I present what might be a feasible solution to both these needs by demonstrating how a relatively small random sample of species can detect a group’s percentage of threatened species as a snapshot while also providing a baseline towards detecting the Red List Index (RLI) trend over time. However, this vision faces several practical challenges; I address issues emerging from taxonomic research in chapter 5, but other obstacles are worth tackling.

6.3.1. The snapshot impossibility

Although I present the proportion of threatened species as a snapshot, there are temporal discrepancies in data availability that make IUCN Red List data unlikely to hold information for a true snapshot, particularly if the species extinction risk assessment is desktop-based and is not ground-truthed (Brummitt et al. 2015a).

It is inevitable that the information needed to assess a species extinction risk takes time to compile. This, in turn, leads to different dates of assessments when publishing them in the IUCN Red List, particularly when assessing large numbers of species for approaches like the sRLI. This entails that our snapshot will, in fact, encompass assessments made throughout a few years rather than a snapshot of a single year. However, this relatively small time lapse is eclipsed by a much greater lag that has not often been considered, the date of the last record, which holds the geographical information that most often informs the IUCN Red List assessment, as criterion B is by far the most used, on which over 50% of species assessments are based (Collen et al. 2016; IUCN 2020).

Clear guidelines that require a time stamp of the last confirmed record of any particular species to be included in future IUCN Red List assessments would be valuable information. These data would enable analysis on which regions, or taxonomic groups, we have not recorded in recent years, how long are current record absences, what is the time lag between recording and assessing, as well as many other metrics that would improve not only transparency but could also guide assessment efforts. However useful these additional data may be, in itself, it would be unlikely to address the root cause of this time lag, the lack of conservation-targeted surveys and species monitoring (Robinson et al. 2018).

In this thesis' case study, most spider species, at the time of assessment, had not been seen in decades or even centuries (Cardoso et al. 2019). Therefore, using available data to produce a snapshot of the group's proportion of threatened species will always be limited by available data. What is needed is for these desktop assessments to inform ground truthing (Brummitt et al. 2015a). This is one of the main challenges we face, as field surveys allowing us to ground truth potential species ranges are scarcely supported financially, particularly towards wide-ranging long-term monitoring. However, field surveys are the only way to truly understand where species are still found today (to produce a snapshot) and establish long-term monitoring programs to understand how they might be doing in the future.

Previous researchers have set a clear path to achieve these goals (Brummitt et al. 2015a), with very insightful approaches to pragmatically address this issue in the face of currently available funding, including prioritising those species more likely to change the IUCN Red List category. I acknowledge that beyond my proposal to prioritise the detection of extinction risk in DD species (chapter 4), focusing monitoring efforts on species with high potential of changing category is a feasible approach to overcome the difficulty of monitoring all species in a random sample (Brummitt et al. 2015a), even if that sample solely contained 200 sp. (chapter 2).

I believe that not only should this be the path ahead towards species conservation monitoring, but it also contains an interesting scientific question under the sampled RLI. In this thesis, I explored which sampling methodology could best capture species more likely to experience IUCN Red List category change. However, overcoming the bias inherent in such a sampled approach was a statistical problem that I was not able to overcome, but one that I believe could be a valuable scientific contribution to the field.

6.3.2. What success looks like

The IUCN Red List currently recommends a waiting period of 5 years before downlisting a species to a lower extinction risk, e.g. when detecting an increase in abundance that would lead to an improvement in species category (IUCN Standards and Petitions Committee 2019). This prevents temporary improvements from being included in the overall trend, and as part of precautionary measures, it fits current scientific practice. However, when aiming to detect a group’s RLI every decade (Henriques et al. 2020), this 5 years waiting period will cause improvements to only be detected 15 years after the initial assessment. Therefore, for improvements to be detected within a decadal inter-assessment period, they have to be detected at a maximum of 5 years after the initial assessment so that a 5-year waiting period can elapse before the measurement of the RLI, 10 years after the first assessment.

This approach towards category improvements, however, justified from a conservation standpoint, may introduce bias in the sRLI. When aiming to defect trend over a feasible ten-year period (Henriques et al. 2020), the second assessment is more likely to capture category decline that took place throughout that entire 10-year period than improvements which took place in the first 5 years of that period. Another way to consider this issue from a pragmatic standpoint is that when reassessing all species in a sample (assuming data availability challenges have been overcome) a decade after assessing them for the first time, all species that declined will be included, while all species that improved will only be included in the next (third) reassessment.

Clear guidelines might resolve this methodological issue by allowing improvements to be included in RLI trend detection even if 5 years have not yet elapsed since particular improvements were detected. However, it is worth noting that this is an issue researchers assessing a random sample every ten years might not face often when measuring a species extinction risk for a second time towards detecting trend direction (Henriques et al. 2020). Instead, improvements are more likely to be found when detecting changes in slope on a species' third assessment, as the average length of time for an improvement in the IUCN Red List category is, on average, just over 16 years (Young et al. 2014).

This 16-year time lapse raises an important underlying issue that deserves further research: not only how fast, but also how effectively can we measure conservation success? To the best of my knowledge, it has not been made clear in the literature if this 16-year time lag (on average) is a methodological issue (caused to some extent by the compulsory 5-year lag mentioned) or simply a reflection of the temporal requirements of species recovery.

Another important issue is the detection of declining species in a random sample and how to address it. In particular, the dilemma between the scientific and moral obligation to support conservation action when encountering declining species versus the bias one introduces if non-random conservation efforts are directed towards randomly selected species (Brummitt et al. 2015a). When considering what success looks like, if our aim is that all species in our sample reverse declining trends (Leclère et al. 2020), but we achieve it by directing conservation action towards threatened species in our sample, it would defeat the purpose of this approach, as this sample would no longer be representative.

Understanding how biodiversity is changing is critical as it underpins human well-being. Still, it is also imperative that we ensure the rich tapestry of the natural world survives for its own right and for future generations to witness. However, biodiversity continues to be lost at an incredible rate, and international targets that attempt to address this continue to be missed (United Nations 2020). However, our picture of this decline is limited, generally focusing on large, conspicuous and well-monitored species. In my thesis, I have tried to show what tools can be applied to understand the extinction risk of a much wider variety of earth’s biodiversity. However, even this process reveals that better data are needed for many species to understand their status.

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6S. Supplementary Materials - No species left behind: What does it take to tackle Data Deficiency in understudied megadiverse groups?

Coelotes amamiensis **Shimojana, 1989 - Data Deficient**

Action taken to address DD: Several taxonomists and local experts were contacted.

Further action needed for DD reclassification: None.

Cause for reclassification: Difference of opinion among assessors.

Taxonomic and biogeographic assessment: As in Shimojana (1989) and Seppälä (2018a).

Relevant information to be added to the species conservation profile: The Ryukyu Archipelago are primarily forested (70%), and this species' rocky habitat is, therefore, naturally fragmented (GFW, 2020). The main threat to *Coelotes amamiensis* is habitat loss due to sea level rise, which is set to greatly impact this archipelago under current climate change scenarios (IPCC, 2014). Climate change is predicted to affect all known sites of similar altitudinal range, and we therefore considered two locations, differentiating between low and high altitudes.

Regular collecting expeditions have failed to detect *Coelotes amamiensis* (Baba pers. comm. 2019), and the species is considered to naturally occur in small populations (Okumura pers. comm. 2019), which places it at greater risk.

These islands host numerous endemic and threatened species, with several threats affecting arthropods, namely the invasive ant *Pheidole megacephala* (Yamauchi & Ogata, 1995), which is known to impact native invertebrate species (Wetterer, 2007) and might compete with or directly prey upon this spider.

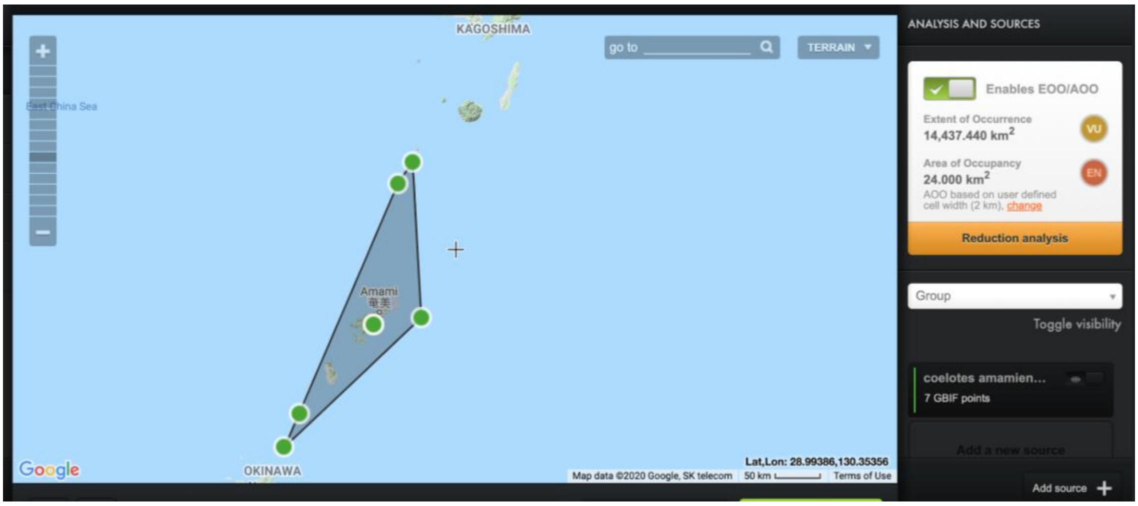
The impacts of invasive ant species on spiders have not been investigated in Japan (Baba pers. comm. 2019), and there are currently no reports on the impacts of *Pheidole megacephala* on Japanese ground spiders. Still, its habitat is slightly different from that of several other coelotine spiders (Okumura pers.

comm. 2019). However, if the invasive species expansion is curtailed, Pheidole megacephala habitat might overlap with Coelotes amamensis, either currently or in the future.

Proposed IUCN Red List Category: EN B2ab(iii)

We consider the preliminary analysis of the *Coelotes amamiensis* to be incorrectly assigned a DD status. For this species to be classified as DD, it would have to be anywhere between Critically Endangered and Least Concern (LC). Under criterion B, its AOO would have to be considerably larger, which we know from the small size of these islands not to be possible (i.e. it is not a marine species). The possibility of LC is made even less likely considering *Coelotes amamiensis* habitat preference and its reduced availability in the region (GFW, 2020). Furthermore, LC status would only be warranted if it inhabited the considerably northwards islands of Yakushima and Taneshima, as well as the much larger Island of Kyushu, where despite recent research on this particular group of spiders (Okumura, 2013), it was not found (Fig. S1). The species would also have to occur considerably southward into the larger island of Okinawa, where it has also not been found despite recent research on this particular group of spiders (Okumura, 2020).

Therefore, although there is very little we know about *Coelotes amamiensis*, with the information currently available, we believe that its extinction risk uncertainty does not merit a DD classification but rather warrants a precautionary approach, which places it in the Endangered category (IUCN, 2019).



☑ Enables EOO/AOO

Extent of Occurrence 14,437.440 km^2 VU

Area of Occupancy 24.000 km^2 EN

AOO based on user defined cell width (2 km). change

coelotes amamien...
7 GBIF points

Figure S6.1 - Geocat analysis of *Coelotes amamiensis,* showing the location of Okinawa to the south and Kagoshima (island of Kyushu) to the north.

Hololena hopi **Chamberlin & Ivie 1942 - Data Deficient**

Action taken to address DD: Validation of GBIF records. Museum collections and several taxonomists and local experts were also contacted.

Further action needed for DD reclassification: This species holotype appears to have been lost (Jiménez and Maya pers. comm) and is so poorly described that it is essentially impossible to identify *Hololena hopi* with confidence. As with many agelenidae and other spider taxa, females are very similar and largely indistinguishable in the absence of associated males (Bennett pers. comm.), and the only existing description lacks important distinctive traits for this group, such as the dorsal part of the epigynum (Jimenez pers. comm.). Furthermore, several characters occur as a gradient, and a number of local variations might prove to be distinct species or subspecies (Hebert pers. comm.).

Therefore, the current data scarcity currently associated with *H. hopi* is likely to be caused by the inability to identify this species with the available taxonomic resources and/or is due to it being a synonym of another species.

No serious effort has ever been made in the last 80 years to define the genus appropriately and provide usable diagnoses and illustrations of verified species. Consequently, the action needed to assess this species is a comprehensive taxonomic revision.

Taxonomic and biogeographic assessment: The preliminary analysis was based on two records, the type locality and a record from South West Region Experimental Station in Cochise County (Seppälä, et al. 2018a). However, this second record was found to be a misidentification of Hololena hola (Ramirez pers. comm.), and the species has not yet been recorded in the area by any of the surveys conducted by the Southwestern Research Station of the American Museum of Natural History (Lanan pers. comm.).

A third record of the species was available on GBIF but was not included in the preliminary analysis as it did not include coordinates. This record was found to be a placeholder for a museum specimen sent on loan (Leibensperger pers. comm.); further investigation of this loan revealed that it was, in fact, the holotype and not a new record (Hebert pers. comm.). The two records now present on GBIF are, therefore, duplicates, and the species, if valid, is only confirmed from the type locality.

Relevant information to be added to the species conservation profile: Additionally to the threats mentioned in the preliminary analysis (Seppälä, et al. 2018a), it is worth noting that several invasive plant species (namely the African buffelgrass, *Pennisetum ciliare*) and the new ecological fire regime they might create, is likely to reduce the species habitat.

Although many areas in the region remain in excellent condition, others face several threats to the habitat of this species, especially the areas with isolated populations in desert canyons that are threatened by mining or urban development (Hebert pers. comm.).

IUCN Red List Category: The possibility of this taxon being a subspecies of another species is at least as likely as the possibility of it being truly range-restricted or inadequately searched for, which would place it as DD “Taxonomic uncertainty explains lack of information”. However, current IUCN guidelines (section 10.3 point 2) state that “taxa should not be classified as Data Deficient simply because of [taxonomic] uncertainty: they should either be regarded as good species and assessed against the Red List Criteria, or not assessed for the Red List” (IUCN 2019).

Since several experts have independently stated that this species does not meet the standards to be considered a good species, we concluded that it cannot be assessed. See chapter 5 for further discussion if it should either be considered Not Evaluated or removed and replaced in the sample.

Oramia occidentalis **(Marples, 1959) - Data Deficiency**

Action taken to address DD: Museum collections, several taxonomists and local experts were contacted, but no further information was acquired. Further enquiries to local stakeholders, such as park rangers, were initiated but interrupted by a global pandemic.

Further action needed for DD reclassification: Taxonomic analysis of closely related species and targeted sampling should allow for the species to be effectively detected if still present in the region and validate its Extent of Occurrence.

Taxonomic and biogeographic assessment: *Oramia occidentalis* was only collected from Whero Island prior to 1959 (Marples 1959). The species might be a variation of a mainland species (*Oramia littoralis*) found 200 km away but appears to be morphologically distinct (Forster & Wilton, 1973), and if proven to be endemic, it would fit speciation patterns in the region.

Relevant information to be added to the species conservation profile: The range of this species appears to be very narrow, with the only known population found exclusively in shallow rocky islands. Sea level rise is set to greatly impact its habitat under current climate change scenarios (IPCC, 2014). Consequently, climate change is predicted to affect all known sites of similar altitudinal range, and we therefore considered one single location.

IUCN Red List Category: CR B2ab(iii)

We consider the preliminary analysis of *Oramia occidentalis* as DD to be unjustified. For this species to be classified as DD it would have to feasibly be anywhere between Critically Endangered and Least Concern (LC). Under criterion B, its AOO would have to be considerably larger, which we know from the small size of these islands to be unlikely (i.e. it is not a marine species). Taxonomic clarification would be important, but sampling efforts in the broader Steward Island region (where the species was found) failed to find it elsewhere, despite appearing to be fairly common in Whero Island (eight specimens were originally collected). In contrast, other species with similar traits were recorded at other nearby sites, namely *Orepukia sorenseni* and *Amaurobius charybdis* (Marples 1959, Forster & Wilton, 1973). This indicates that it might indeed be limited in range.

Amaurobius transversus **Leech, 1972 - Data Deficient**

Action taken to address DD: Several taxonomists and local experts were contacted.

Further action needed for DD reclassification: None needed.

Taxonomic and biogeographic assessment: The type locality of the species was described as “Redwood Canyon”, which is also a clear clue to the species habitat, Redwood forest. This particular canyon has since been renamed Nathaniel Owens Memorial Redwood Grove and is now one of the largest remaining stands of this habitat.

The fact that this species has not been seen since description might lead to the assumption that it is either a single site endemic or that it is an invalid species (Hedin pers. comm.). However, we think it is also worth noting that the same author described a species from the same group, based on 20 adult female specimens and 30 immatures (*Amaurobius diablo)* from four Californian Counties. Yet, the species remains unrecorded since description as well. Therefore, we cannot exclude the possibility that the species may be valid and may occur in several localities but (similarly to A. diablo) still remain undetected as well due to crippling under-sampling of this particular Californian region.

Relevant information to be added to the species conservation profile: As an inhabitant of redwood forests, forest clear-cutting is a major threat to this species’ habitat in the region (Hedin pers. comm.). This threat can be regarded as reduced in the sections of this habitat that are legally protected. However, the current political situation in the United States has made the future protection of several of these habitats as national monuments uncertain (Britton-Purdy, 2018). Furthermore, 21,232 fires were recorded in California within a year (March 2019-March 2020), 170 to 405 of which took place in Monterey County (where this species was recorded) during that same period (GFW, 2020).

The region is now home to an invasive Australian spider, *Badumna longinqua* (Fig. S2), which was first recorded in California in 1918 (Leech, 1972). One hundred years later, the species is now widely spread in the state and very common (Henriques pers. obs.). *B. longinqua* is a generalist predator (Kennedy et al. 2020). It has been recorded feeding on other spiders, potentially as a behavioural and/or dietary shift in their invasive range (Viera et al. 2019).

Although phylogenetically very distinct, this invasive species is morphologically so similar to Amaurobius transversus that it was originally described in the same genus (*Amaurobius longinquus*). Therefore, although nothing is known about the behaviour of *A. transversus* or its overall biology, if the two species' ranges overlap, they are likely to compete for habitat and prey, and *B. longinqua* might even attack or kill *A. transversus.*

At the time of *A. transversus* description, no record of *B. longinqua* was made in the same area, but the invasive species was already present nearby (Leech, 1972) and is likely still expanding.

IUCN Red List Category: Should remain DD, although targeted surveys might exclude the possibility of LC and even warrant the classification of CR B2ab(iii).

Figure S6.2 - The Australian spider, *Badumna longinqua*, was introduced to the U.S. a century ago and is now common in coastal California.

***Callobius pauculus* Leech, 1972 - Data Deficient**

Action taken to address DD: Reanalysis of GBIF records were paramount towards contacting relevant Museums and becoming aware of local experts.

Further action needed for DD reclassification: None needed.

Cause for reclassification: Available data was missed in the preliminary assessment.

Taxonomic and biogeographic assessment: Besides the type locality, a second record was later found from Mendocino National Forest Road (Seppälä, et al. 2018a, GBIF, 2020). This unanalysed record was linked to an in-depth analysis of *Callobius pauculus* (and its closely related species, C. Paskenta), which concluded that both species are narrow endemic species, both with identical microhabitats in Pinus dominated forests (Lew, 2011). However, *C. pauculus* inhabits the highest peak areas, whereas *C. Paskenta* inhabits lower elevations (Lew, 2011).

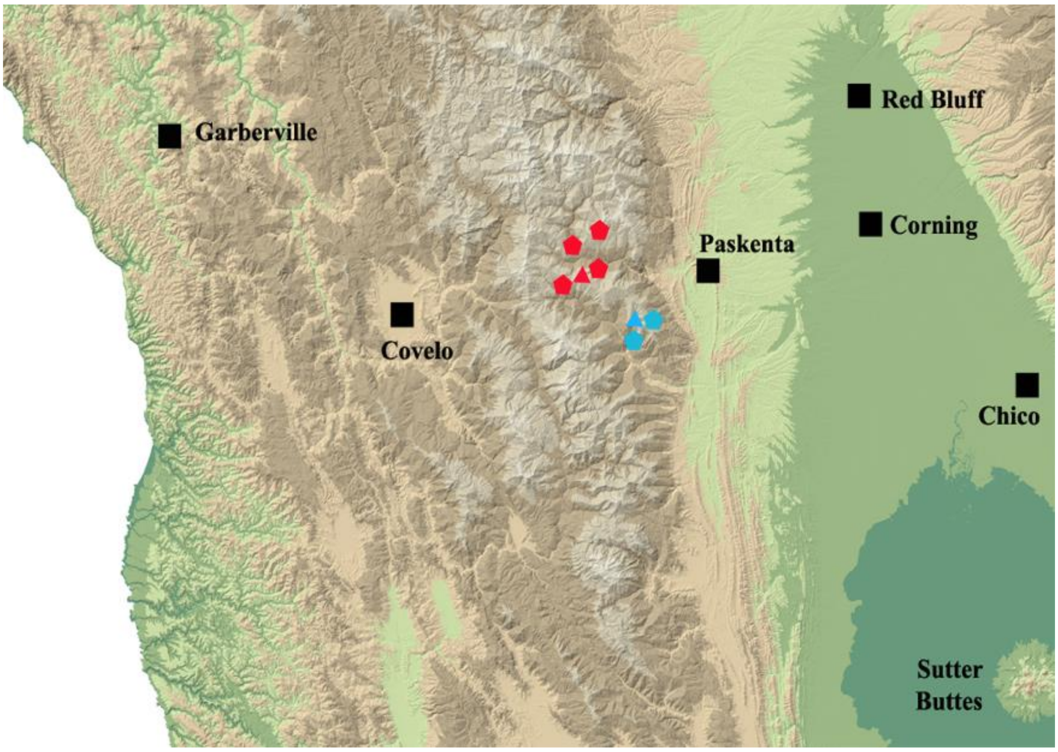


Figure S6.3 - Range of *Callobius pauculus* in red shapes and *Callobius paskenta* in blue shapes. Black squares indicate urban centres. Triangles represent the type locality (Leech, 1972) and are mentioned in the preliminary analysis (Seppälä, et al. 2018a). Pentagons represent the unaliased records mentioned here.

Relevant information to be added to the species conservation profile: Similarly to other Amaurobiidae that are mostly ground-dwelling spiders (Platnick, 2020), *Callobius pauculus* is more often found under loose bark or occasionally other debris on the ground, but never under rocks (Lew pers. comm.). This species builds simple silk retreats similar to other members of the genus, with a characteristic hackle-mesh (lacking symmetry or clear structure), which is often composed of a single wide chamber surrounded by a thin layer of cribellate silk (Lew pers. comm.).

Callobius pauculus belongs to a clade of California Floristic Province neoendemic species, seems to occur in low density populations and inhabits a very narrow range in high mountain peaks (Lew, 2011), not temperate forests as initially assumed (Seppälä, et al. 2018a). The elevation range of the type locality was considered to be 1376 m (Seppälä, et al. 2018a), but current literature estimates that specific record to be approximately 1500 meters (Lew, 2011).

This spider is considered to be sensitive to climate change due to its narrow range and because these high montane forests are expected to be impacted by warming and drying under climate change scenarios (Rangwala and Miller 2012, Foden et al. 2013, IPCC 2014). This species is known to occur between 1100-1800 m of altitude (Lew pers. comm.). Climate change is also likely to increase the probability of large fires in northern California, further endangering this species’ habitat (Syphard et al. 2019). One single location is considered since the species occurs only in this narrow high-altitude threshold, and the loss of habitat by climate change should impact all known populations simultaneously.

Furthermore, although the species range falls within the Mendocino National Forest Road, it is managed by the United States Forest Service, which is part of the United States Department of Agriculture. Hence, logging and housing development can occur legally, both of which could destroy this species' entire range in a single action, and even if not directly destroying the habitat, housing and human infrastructure are consistently linked to fire ignitions in California (Syphard et al. 2019).

IUCN Red List Category: CR B2ab(iii)

Thaloe ennery **Brescovit, 1993 - Data Deficient**

Action taken to address DD: The taxonomist who described the species was contacted, as well as local experts who were aware of other material of this species in museum collections.

Further action needed for DD reclassification: None needed.

Cause for reclassification: Available data was missed in the preliminary assessment.

Taxonomic and biogeographic assessment: The extant arachnofauna of Hispaniola is recognised as poorly known (Penney & Pérez-Gelabert, 2002) but since the preliminary analysis (Seppälä, et al. 2018a), seven further records were found to exist, spanning from the early 1990s to 2019 (Carrero in press).

Relevant information to be added to the species conservation profile: The species has been recorded in tropical and subtropical moist broadleaf forests with some calcareous outcrops (Seppälä, et al. 2018a) both in Haiti and the Dominican Republic, but wandering males have also been detected in the highland savanna of *Danthonia domingenis* grassland (Carrero in press).

The females of *Thaloe ennery* have never been recorded, while mostly single males (only one occurrence recorded with more than one specimen) have been found from late March to mid-August, and one record was made in late September (Carrero in press.).

Thaloe ennery altitudinal range spans from just above sea level to more than 2,200 m asl (Carrero in press.). The main threat across its range is habitat loss due to severe deforestation. In the Dominican Republic, deforestation is caused by the illegal manufacturing of charcoal and illegal crop production (Carrero in press.). Tourism development has been particularly impactful on the coast (MMARN 2011), where several of this species' records were made, and unsustainable tourism practices and consequent land exploitation (without proper management/permission) have already severely impacted at least one of the localities where *T. ennery* has been found in the past (Carrero in press.).

It is estimated that only 33% of the Dominican Republic's vegetation cover area remains (MMARN 2011), with c. 250,000 ha of forest lost between 2001 and 2016 (World Resources Institute 2018) and a percentage of total land area covered by natural forest reduced from 77% in the 1920s to 13% in 1980s (Davis et al. 1997).

Hurricanes are another threat in Hispaniola (Davis et al. 1997, Sherman et al. 2001, Moreno & Ocampo 2011), which will become more frequent with climate change (MMARN 2011, Moreno & Ocampo 2011). An increase in temperature, reduction of rain volume and sea level rise are other impacts of climate change predicted for the Dominican Republic (MMARN 2011).

Furthermore, there are more than 130 invasive species reported from the Dominican Republic (MMARN 2011).

An analysis of known records produces an EOO of 32,656 km2 and an AOO of 32 km2. However, the species has not been seen in Haiti for more than 85 years, and deforestation in the country is so severe that this tree-dwelling species is unlikely to still occur in the region. Therefore, we removed this Haitian record from our analysis and considered an EOO of 11,176 km2 and an AOO of 28 km2, representing an EOO reduction of 66% and an AOO reduction of 13%.

Several of the species records occur within protected areas, but the use of fire to clear land for agriculture and pastures is a common practice, including inside these areas (MMARN 2012).

Therefore, we have considered seven locations, one for each of the currently known records, where the species is likely to still occur.

<u>IUCN Red List Category:</u> VU B1ab(i,ii,iii,iv)

Timbuka meridiana **(Koch, 1866) - Data Deficient**

Action taken to address DD: Local experts were contacted, who were aware of other material of this species in museum collections.

Further action needed for DD reclassification: Taxonomic analysis.

Taxonomic and biogeographic assessment: *Timbuka meridiana* is a prime example of an early description that has been poorly illustrated, in a region where the group is highly diverse (Martinez pers. comm.) and the lack of a modern taxonomic analysis hinders the understanding of the group's true diversity.

The original type locality is considered unreliable, as the collectors received specimens from a diverse set of nearby regions and countries, which have often been published as originating from Bogotá when they were not collected there (Galvis pers. comm.).

Several potential records of this species have been made in the Cundinamarca and Boyacá departments (Martinez pers. comm.) which have not been included in the preliminary analysis of the literature (Seppälä, et al. 2018a). The revision of the type material and the upcoming verification of these records will likely help shed some light on the species' current distribution (Martinez and Galvis pers. comm.). They should be supported as the most relevant course of action to ascertain the species extinction risk.

IUCN Red List Category: Should be reclassified as Not Evaluated until the Natural History Museum, London, reopens to scientific visitation (currently closed due to the global pandemic), as this is where the holotype is held. The analysis of the holotype and the photographic record of its morphological feature will allow for available records of this species to be disambiguated with certainty from closely related species, enabling its range to be measured, relevant threats analysed, and its extinction risk category to be assessed under the IUCN Red List criteria.

Araneus camilla **(Simon, 1889) - Data Deficient**

Action taken to address DD: Local experts were contacted.

Further action needed for DD reclassification: Taxonomical analysis and targeted sampling should effectively detect the species' presence in the region and validate its range.

Taxonomic and biogeographic assessment: In our preliminary analysis, *Araneus camilla* type locality was considered to be Delta near New Dehli (Seppälä, et al. 2018a). However, further analysis of this record showed that the type material of this species originated in Deota, Janosar (aka Jaunsar-Bawar) in present day Uttarakhand state (Caleb pers. comm.) in the west Himalaya (Caleb & Kumar, 2018).

The second record of *Araneus camilla* gathered by our bibliographical analysis, from Lahore in Pakistan (Seppälä, et al. 2018a) is currently considered to be a misidentification (Caleb pers. comm.). All records from that historical publication (Dyal, 1935) should be viewed with a substantial dose of scepticism, as that analysis listed species to Pakistan that are known to be endemic to the Americas (Ali pers. comm.).

Unfortunately, the specimens from that seminal Pakistani collection (Dyal, 1935) are not currently preserved in any University or Museum in Pakistan (Ali pers. comm.); hence, the identity of the material that originated this record cannot be confirmed.

This Pakistani record of *Araneus camilla* was made without the study of the type material, and identification was likely based solely on the original text (Caleb pers. comm.), which does not provide a detailed description of *Araneus camilla* but just distinguished this species from *Gibbaranea bituberculata* (as *Epeira dromaderia* Walckenaer, 1805). This does not enable the correct identification of the species.

Therefore, we have excluded the Lahore record of this species from our analysis and consider this species to be endemic to the west Himalaya region (Caleb & Kumar, 2018).

IUCN Red List Category: Should remain DD, but the present revised “desktop” assessment should now enable us to more accurately “ground truth” the species range, as proposed by other researchers (Brummit et al. 2015b).

Cyrtarachne hubeiensis **Yin & Zhao, 1994 - Data Deficient**

Action taken to address DD: Taxonomic experts were contacted.

Further action needed for DD reclassification: Taxonomic analysis of closely related species and targeted sampling should allow for the species to be effectively detected if still present in the region and validate its Extent of Occurrence.

Taxonomic and biogeographic assessment: No further records of this species were retrieved, but we found this species to be another example of an insufficient taxonomic description that hinders the validation of further records of this species, even if it is collected for analysis. Preliminary analysis of available bibliographical data indicates it is likely a valid species, differing from *C. inaequalis* type material, which is in turn similar to *C. fangchengensis* (Tanikawa pers. comm.), two species which might potentially be synonymised. The bibliographical records of the genus in China, including *C. hubeiensis*, *C. bufo, C. fangchengensis* and *C. inaequalis* need to be taxonomically revised (Tanikawa pers. comm.), and the current literature is so unclear that even the reference global catalogue of spiders has misinterpreted these species ranges (WSC, 2020).

Our in-depth review of these species records in East Asia (Fig. S4) indicates that there is potential range overlap between species, increasing the likelihood of misidentified records. Fig. S4 shows the region where each species has been reported from in all available literature and should be used to inform where it would be most suitable to conduct targeted samples to find these animals.

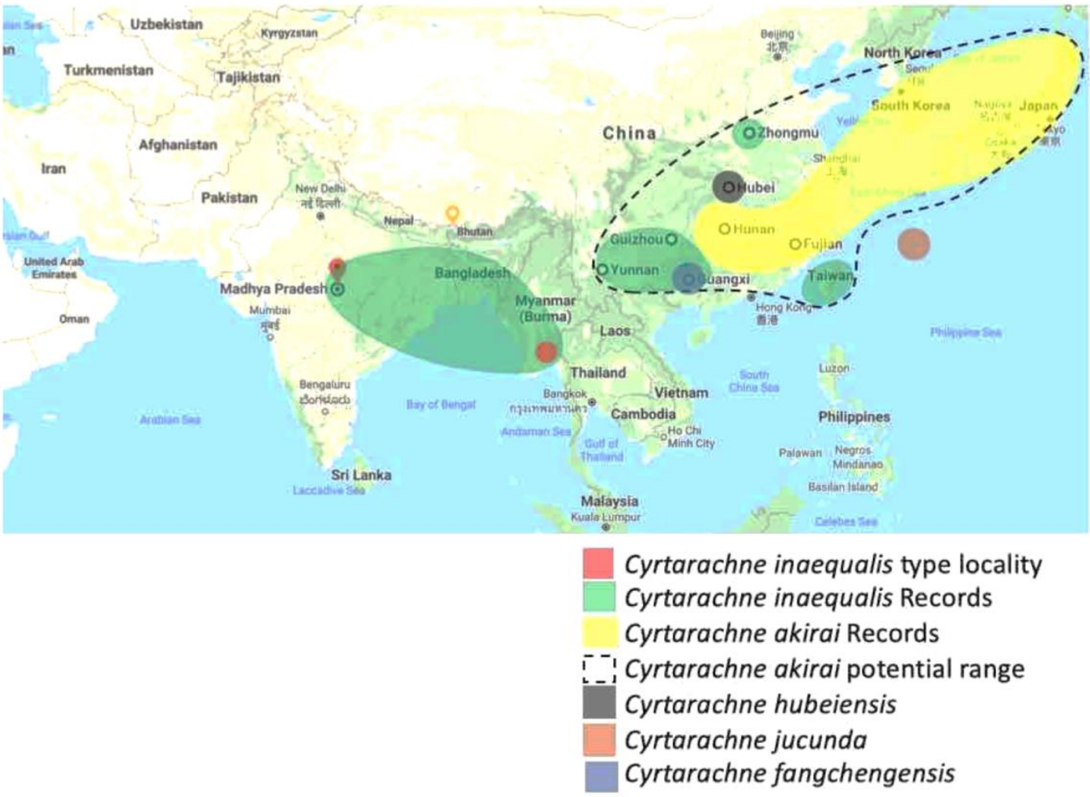


Figure S6.4- Distribution of *Cyrtarachne hubiensis* and closely related species.

<u>IUCN Red List Category:</u> Should remain DD since it can as likely be Least Concern as Critically Endangered. At present, the revised “desktop” assessment does not allow us to disambiguate if other records of this genus in the region might belong to this species or not. Therefore, as in so many more species from our sample, a taxonomic revision of available specimens is the first step before considering “ground truthing” the species range, as proposed by other researchers (Brummit et al. 2015b).

Tatepeira itu Levi, 1995 - Data Deficient

Action taken to address DD: Local taxonomic experts were contacted.

Further action needed for DD reclassification: None needed.

Cause for reclassification: Available data were missed in the preliminary assessment.

Taxonomic and biogeographic assessment: The species has not been recorded from the northernmost record of its range since 1959 (a historical farm which receives regular visitation from the general public) and has not been recorded in one of the most studied areas in the country, Sao Paulo city since 1945 (Levi, 1995). However, this remarkably long absence of records might result from undersampling (Brescovit pers. comm.).

Nevertheless, fairly recent records (from 2000 and 2003) have been made near the southernmost record of this species in two of the few remaining small patches of natural vegetation in that area (Ott pers.comm.).

The main threat to this species appears to be habitat loss due to deforestation by urban development, agriculture, livestock farming and mining. It is estimated that only 11.4% to 16% of the original Atlantic Forest vegetation remains and c. 42% of the total forest area is represented by fragments smaller than 250 ha (Ribeiro et al. 2009). Brazil's most populated urban centres and the largest industrial and silvicultural centres are found within the original area of the Atlantic Forest (Critical Ecosystem Partnership Fund 2001). Although the deforestation rate has been decreasing in the last few years, it is still ongoing. Forest area and quality have been continuously declining for at least the last 30 years, with remaining areas of natural vegetation as low as 7.9% in Rio Grande do Sul and as high as 22.9% in Santa Catarina (Fundação SOS Mata Atlântica and INPE 2019).

IUCN Red List Category: CR B2ab(iii)

Wagneriana yacuma **Levi, 1991 - Data Deficient**

Action taken to address DD: Bibliographical sources were reanalysed.

Further action needed for DD reclassification: None needed.

Cause for reclassification: Available data were missed in the preliminary assessment.

Taxonomic and biogeographic assessment: Named after the Yacuma River in Bolivia, where it was originally found in 1954 (Levi, 1991), this species was also recorded from Brazil's Mato Grosso and Mato Grosso do Sul a few years after its description (Buckup & Pinto-da-Rocha 1996). Its Extent of Occurrence has been estimated as 303,496 km2, and it has been reported as one of the most common araneids in the flood forests of Miranda (Raizer, 2004). This species also appears to tolerate some level of human disturbance (recorded in considerable numbers at a farm in Pocomé by Buckup & Pinto-da-Rocha 1996, and recorded in the outskirts of Cuiabá in 2015 by Cabra-García & Hormiga, 2019). Consequently, this species appears to be Least Concern, but due to the small number of locations (<10), severe habitat loss and likely loss of AOO, it is proposed to be placed as NT.

IUCN Red List Category: NT B2ab(iii)

Cardiopelma mascatum Vol, 1999 Data Deficient

Action taken to address DD: Local taxonomic experts were contacted, and reanalysis of GBIF records was paramount towards gathering data and finding further local experts.

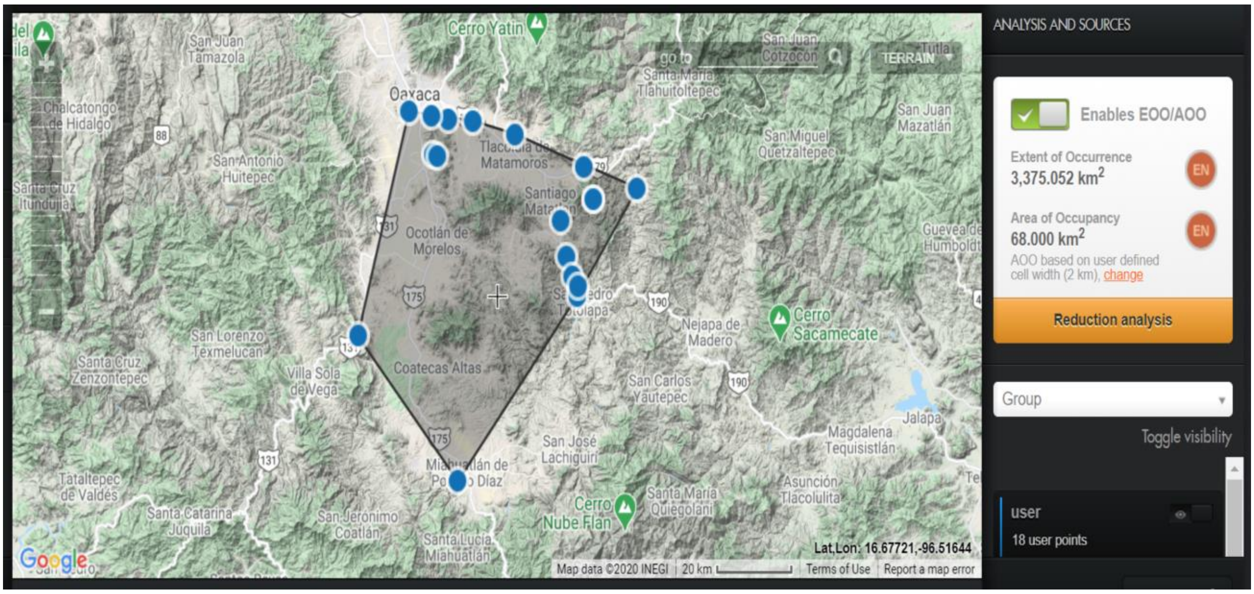
Further action needed for DD reclassification: None needed.

Cause for reclassification: Available data were missed in the preliminary assessment.

Taxonomic and biogeographic assessment: The sole record considered during the preliminary analysis was the original description (Seppala et al. 2018d) based on a specimen from the pet trade, with indications the species might be from Mexico. A published record of the species was available at the time (Mendoza et al. 2016) but was missed (see comments below). A GBIF record of this genus existed but contained no georeferenced data while proving a connection to its bibliographical origin (Ortiz et al. 2018). Because this is a monospecific genus, we considered it warranted in-depth analysis, which revealed to contain two georeferenced records from Oaxaca, Mexico, solely identified to the genus level (Ortiz et al. 2018), one of which could confidently be assigned to *Cardiopelma mascatum* (Mendoza pers. comm.). Several other unpublished records of this species also existed, which allowed for a reassessment of its extinction risk category (Mendonza, Longhorn and Locht pers. comm.)

Cardiopelma mascatum occurs in undisturbed habitat where available rocks provide shelter for multiple individuals in adjacent and even shared silken tunnel burrows (Goyer pers. comm.). Some of the sites where the species was recorded had observable soil degradation caused by cattle ranching and local farming, where, despite these conditions, several individuals still thrived (Goyer pers. comm.).

IUCN Red List Category: LC Although the Extent of Occurrence of this species fits the Endangered threatened category, it does not seem to be impacted by any major threats at present. Despite being present in the pet trade, the impact of current poaching levels is unknown, and as noted, it can thrive in disturbed habitats. Consequently, the number of locations is not applicable, and the species does not appear to be severely fragmented; therefore, it should be assessed at present as LC. If the species proves to be dependent on rocky environments, severe fragmentation might have taken place, and a threatened category may be granted. In this case, connectivity might potentially be restored if stone walls or closely spaced stone piles are encouraged as a conservation action.



Extent of Occurrence 3,375.052 km^2 EN

Area of Occupancy 68.000 km^2 EN

AOO based on user defined cell width (2 km), change

user

18 user points

Lat,Lon: 16.67721,-96.51644

Figure S6.5 - Geocat analysis of known and unpublished records of *Cardiopelma mascatum.*

<u>Further comments:</u> When conducting the preliminary assessment of this species (Seppala et al. 2018d), the used methodology (such as the academic search engine Google Scholar) found no further publication with valid records beyond the original publication (WSC, 2020). However, other publications containing the only published geographical record of the species were available at the time (Mendoza et al. 2016) but were not easily accessible. This species, therefore, highlights the importance of retrievable information and data sharing, as current practices might prevent researchers from accessing valuable published data.

It is also worth noting that several closely related species await description, and among the species of *Cardiopelma* so far analysed, *C. mascatum* is the one with the largest range of distribution (Mendonza, pers. comm.). This example perfectly illustrates how currently described species are more likely to be the ones with the largest range and be more common overall, hence more likely to be listed as LC.

Whereas species with smaller ranges are more likely to remain undescribed and, therefore, cannot be included in a randomly selected sample if it is solely based on currently described species (see chapter 5).

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Ben, I still can't believe you are gone; just writing feels surreal. I still can't accept we just don't have more time. I miss our meetings, miss your advice, I even miss your jokes. I miss you Ben. Thank you for always caring about me, for truly listening, for grasping what I was trying to say and for helping me say it clearer and stay on track. You taught me so much, yet I now don't know what to say. You were always the one who was good with words, not me. I could have really used your help writing this right now. Although you can no longer advise me, every time I am lost and don't know what to do, I will ask: What would Ben do? Thank you Ben, wish you were here to see this. Ben Collen (12 February 1978 - 19 May 2018).

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