The development of a protocol for declaring alien species absent from South Africa.

by

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Abstract

Invasive alien species are a major driver of ecological threat globally. Effective conservation efforts can be hindered by inaccurate lists of alien species. This study addresses the challenge of inaccurate alien species lists, specifically focusing on the issue of disputed record regarding the presence or absence of certain species in a given region. These disputed records contribute to uncertainties in conservation and management efforts, as species may be erroneously regulated or overlooked based on conflicting data and interpretations. To address this, the study develops a protocol for systematically assessing the presence or absence of alien species. It introduces a robust protocol for declaring the absence of alien species, addressing concerns raised regarding the need for clear evidence in absence declarations. Emphasising evidence-based lists as foundational for strategic conservation efforts, the protocol, rooted in literature reviews and argument maps, classifies species based on their historical likelihood of being present and the probability of them being no longer present given they were once present. The study's methodology, encompassing identification certainty, evidence quality, temporal evidence accumulation, population loss likelihood, search effort, and ongoing introductions, enhances our understanding of species persistence. Applied to Chilean black urchin [*Tetrapygus niger* (Molina, 1782)], the protocol demonstrates its worth as a valuable tool, integrating detailed species information, assessment data, rationale behind the absence/presence assessment and a probability scoring system to provide clear justification for the conclusions drawn. Based on this, I suggest the removal of *T. niger* from regulatory lists in South Africa. This demonstrates the protocol's simplicity, flexibility, and applicability, particularly in situations where data is limited. To test the usability and efficacy of the protocol, it was applied to two additional case study species with disputed presence data in South Africa, *Calluna vulgaris* and *Euphorbia esula*. Based on the proposed probability scoring guide, *C. vulgaris* resulted in a medium probability of absence necessitating further investigation, while *E. esula* was pronounced likely to be absent. Validation through field observations for *C. vulgaris* and *E. esula* confirms the protocol's efficacy as *C. vulgaris* was found and *E. esula* was not. Continuous monitoring is thus crucial, especially for cases with a medium probability score, indicating ongoing uncertainty. The results emphasise the protocol's usefulness but also acknowledges inherent limitations and context-specific dependencies. The study emphasises the importance of recent, verified field observations for accurate list and highlights the significance of robust data collection for effective conservation management. The implications also underscore the importance of methodological rigor in search efforts and survey methodologies. The adaptation of the approach to address mobile species is anticipated, and the development of databases capturing search efforts is recommended. This protocol holds potential in enhancing the precision, transparency, and uniformity of alien species presence/absence

determinations. However, it should be viewed as a targeted tool tailored for specific contexts, such as ecosystems with limited data availability or disputed species records, rather than a universal solution to the challenges related to alien species management and conservation

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Abbreviations and Acronyms

Abbreviation/Acronym	
IAS	Invasive alien species
NEM:BA	National Environmental Management: Biodiversity Act (Act No. 10 of 2004)
NEM:BA A&IS Regulations	National Environmental Management: Biodiversity Act (Act No. 10 of 2004): Alien and Invasive Species Regulations
SANParks	South African National Parks
SAPIA	Southern African Plant Invaders Atlas

Glossary

Term	**Definition**

Alien species	A species that is present in a site outside its natural range as a result of human action that has enabled it to overcome biogeographic barriers.
Detection	The process of observing and documenting an invasive species.[2]
Eradication	The complete and permanent removal of all individuals and propagules of a population of an alien species from a particular site by means of a time limited campaign to which there is a negligible likelihood of reinvasion.[1]
Impact	The effect of an alien species on the physical, chemical, and biological environment. It can include both negative and positive effects.
Introduced	See Introduction.
Introduction	The movement of an alien species (either accidentally, intentionally and legally or intentionally and illegally) by human activity to a region outside its native range.
Invasion debt	The potential increase in biological invasions at a site over a particular time frame in the absence of any interventions. It is composed of the number of new species that will be introduced (introduction debt), the number of species that will become invasive (species-based invasion debt), the increase in area affected by invasions (area-based invasion debt), and the increase in the negative impacts caused by introduced species (impact-based invasion debt).[4]
Invasion science	A term that describes "the full spectrum of fields of enquiry that address issues pertaining to alien species and biological invasions". Invasion sciences includes invasion ecology, but also encompasses non-biological lines of enquiry, such as economics, ethics, sociology, and inter- and transdisciplinary studies.[3]
Invasive alien species	Alien species that sustain self-replacing populations over several life cycles, produce reproductive offspring, often in very large numbers at considerable distances from the parent and/or site of introduction, and have the potential to spread over long distances.
Invasiveness	The characteristics and features of an alien species, such as their life-history traits and modes of reproduction, that define their ability to invade or become invasive.
Lag phase	The time between when an alien species arrives in a new area and the onset of the phase of rapid, or exponential, increase.[5]
Native species	Species that are found within their natural range where they have evolved without human intervention (intentional or accidental). Also includes species that have expanded their range as a result of human modification of the environment that does not directly impact dispersal (e.g. Populations are still considered native if they result from an increase in range as a result of watered gardens, but are considered alien if they result from an increase in range as a result of spread along human-created corridors linking previously separate biogeographic regions).
Naturalised	Alien species that sustain self-replacing populations for several life cycles or over a given period of time without direct intervention by people or despite human intervention.

Pathways	A broadly defined term that refers to the combination of processes and opportunities that result in the movement of alien species from one place to another. A combination of activities and opportunities that result in the movement and introduction of alien species from one place to another.
Permit	An official document issued in terms of Chapter 7 of National Environmental Management: Biodiversity Act, 2004 (Act no. 10 of 2004).
Risk analysis	The process of identifying and assessing the likelihood and consequence of an event, as well as considerations as to how to manage and communicate the risk.
Risk assessment	The process of formally evaluating the likelihood and consequence of an alien taxon by assessing its potential impact on the local environment, ecosystem dynamics, and native species' populations. It forms part of risk analysis, a broader process that involves identifying, assessing, managing, and communicating risks.
Stowaway	The accidental introduction of an alien species attached to or within a transport vector or their associated equipment and media. The organism is transported by chance, and there is no specific, natural association with the vector.
Taxon (plural taxa)	A group of organisms that all share particular properties (usually evolutionary history). The grouping can be below, at or above the species level.

*Definitions used from the report "the Status of Biological Invasions and their Management in South Africa 2019" (Zengeya & Wilson, 2020) except for where indicated by a superscript number: [1]Genovesi, 2011; [2]Reaser et al., 2020; [3]Richardson & Pyšek, 2008; [4]Rouget et al., 2016; [5]van Wilgen et al., 2020.

Chapter One:

1.1. Introduction

The introduction of species beyond their native ranges stands as one of the primary driver of global environmental change (Ricciardi, 2007). The introduction of invasive alien species can result in a number of adverse ecological impacts on the environment (Jeschke et al., 2014; Ricciardi et al., 2013). These impacts may include a reduction in the prevalence and establishment of native species, interference with natural ecological processes like the cycling of nutrients or hydrology, and a reduction in the resources available to wildlife, including food and habitat (Pyšek et al., 2012; Weidlich et al., 2020; Erckie et al., 2022). Over centuries, humans have introduced various species outside of their natural ranges, both intentionally and unintentionally (Seebens et al., 2017). In the coming decades, it is anticipated that the international exchange of alien species (i.e. species introduced to habitats outside their native distribution range due to human activities) will continue to increase steadily, leading to new invasions all over the globe (Bradley et al., 2012; Seebens et al., 2021; Turbelin et al., 2017). However, the probability of a species becoming invasive after introduction is generally fairly low (Smith et al., 1999). Most introductions either fail to establish or if they do establish, they struggle to survive and never naturalise and form invasive populations (Lodge, 1993; Williamson & Brown, 1986; Williamson & Fitter, 1996; Essl et al., 2017). A population is only considered invasive once it is able to successfully overcome an array of biotic and abiotic barriers (Blackburn et al., 2011). These barriers consist of introduction, captivity or cultivation, survival, reproduction, dispersal, and interactions with the local environment and biota (Blackburn et al., 2011). Notably, only a small portion of introduced alien species will form naturalised populations and a portion of those species with naturalised populations will form invasive populations (Richardson et al., 2000, Blackburn et al., 2011).

1.2. Pathways of introduction

Alien species can be introduced into an area either intentionally or unintentionally (Levin, 1989). According to van Wilgen et al. (2020), unintentional introductions are on the rise and that there is frequently a substantial time delay between the unintentional introduction and detection (Faulkner et al., 2020a). For example, in Bush et al. (2016) it was reported that six insects native to Australia were recorded for the first time in South Africa between 2012 and 2014 within a *Eucalyptus* plantation after many years of *Eucalyptus* trees being imported to the country for forestry. It was assumed that the insects were unintentionally introduced along with the trees as contaminants but only discovered many years later (Hulme et al., 2008). Another example is the assumption that the larvae of Chilean black urchin (*Tetrapygus niger*) were accidently introduced to an onshore oyster farming facility in

Alexander Bay, South Africa with the oyster spat from the Pacific oyster (*Crassostrea gigas*) (Haupt et al., 2010, Mabin et al., 2015). The quilted melania (*Tarebia granifera*), a sea snail unintentionally introduced into South Africa as a stowaway through the aquarium trade, escaped these aquarium facilities and invaded a number of rivers, lakes, wetlands, and estuaries in the country (Appleton et al., 2009). There are also alien species that were intentionally introduced, spread, and became invasive. Many intentionally introduced alien species are economically valuable (cf. Williams, 1981; DiTomaso et al., 2010, Richardson & Rejmánek, 2011; Wosiwoda et al., 2014; Hirsch et al., 2020), yet they may have harmful effects on native populations, species, and communities (Ewel et al., 1999; Mack & Erneberg, 2002). For example, alien freshwater fish species like brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), smallmouth bass (*Micropterus dolomieu*), largemouth bass (*Micropterus salmoides*), and Nile tilapia (*Oreochromis niloticus*) were originally intentionally introduced to some South African waterbodies for angling (Cambray, 2003; Ellender & Weyl, 2014). Subsequently these fish became conflict of interest species as they are of importance for anglers, but are also preying on native fish species and causing local extinctions (Ellender et al., 2017; Weyl et al., 2020). Similarly, the increase of ornamental fish-keeping is also linked to increased alien fish species in both marine and freshwater environments (Lockwood et al., 2019; Sandström et al., 2014). Intentional introductions through online sales and trading of species pose new biosecurity threats (Duggan, 2010; Faulkner et al., 2020b; Padilla and Williams, 2004), particularly in the pet, aquarium, and ornamental plant trades (Gaertner et al., 2016). Species brought in for these purposes can establish and become invasive (Dorcas et al., 2012; Henttonen & Huner, 2017; van Wilgen et al., 2010; Alston & Richardson, 2006; Bowers et al., 2006; Pyšek et al., 2012) because escaped or released pets contribute to new alien populations (Kenis et al., 2007; Hulme et al., 2008). These trends raise concerns about unregulated introductions and their potential invasiveness and highlight the need for accurate alien species lists (McNeeley, 2001; Lockwood et al., 2019). To ensure effective management of alien species, accurate species lists, and concomitant legislation is critical.

1.3. Alien species lists

Alien species lists are foundational for national (and regional) programs dealing with invasive alien species management (Genovesi et al., 2010). To ensure that management efforts are focused on effectively addressing all species, each country must maintain an up-to-date inventory of alien species (Brenton-Rule et al., 2016; Genovesi & Shine, 2004; Latombe et al., 2017; McGeoch et al., 2012; Pyšek et al., 2012; Regan et al., 2002). Alien species lists are important as they inform the prioritisation of control and eradication initiatives (Tollington et al., 2017; Bertolino et al., 2020) as well as assist in preventing future introductions (Maceda-Veiga et al., 2019). The accuracy of these alien species lists, along with the introduction status data of alien species, is critical for both the region for which they are compiled, as well as the rest of the world (Wilson et al., 2011; Latombe et al., 2017). To maintain the accuracy of alien species lists, updates are essential to incorporate the latest definitions, nomenclature, and evidence of species presence, absence or potential absence (Richardson et al., 2020). This ongoing process aligns with regulations and practices aimed at enhancing list precision, as is evident from EU Regulation no. 1141/2016, the work of Pagad et al. (2018), contributions from the Eurogroup for Animals (2020), and Toland et al. (2020). These sources collectively demonstrate the concerted efforts to enhance the accuracy and reliability of alien species lists through continuous updates and adherence to evolving standards and practices. Over the last decade, significant progress has been made in collecting data about alien species and their distribution around the world (Dyer et al., 2017, van Kleunen et al., 2019; Biancolini et al., 2021). Updates take place as new information becomes available, when individuals and organisations in responsible for maintaining the lists change, and as taxonomists revise classification of taxa (Briggs & Leigh, 1996). For example, Czechia's total alien flora increased to 1 454 taxa in 2012 (Pyšek et al., 2012a) from 1 378 taxa in 2002 (Pyšek et al., 2012b). This increase by 76 taxa was a result of a meticulous examination and re-evaluation of taxonomic literature, herbaria, and various other sources in addition to the increase of newly identified taxa during decade prior (Pyšek et al., 2012a). This highlights the difficulty in maintaining updated and accurate alien species lists.

Alien species lists can be very complicated to compile and are not devoid of errors. Errors in alien species lists can inflate species counts, cause confusion, and result in wasted or ineffective management effort. Accurate alien species lists, including presence/absence status, are vital for managing and monitoring alien species (McGeoch et al., 2016) and preventing future introductions (McGeoch et al., 2010, 2012). There are however typically several types of errors, biases, and uncertainties that exist within these lists (Magona et al., 2018; McGeoch et al., 2012). Lists of alien species may have inaccuracies and significant errors (Burgman, 2004), but the actual rates of these errors are unknown (Richardson et al., 2020). The causes for inaccuracies in these alien species lists include, but are not limited to, insufficient available information, under-sampling of species, low investment in invasive alien species (IAS) research, and time delays between the identification of a new alien species and publication in national species lists (McGeoch et al., 2010). Uncertainties may become apparent as a result of flawed detections, erroneous recordings (Guillera-Arroita et al., 2014; Yoccos et al., 2001), knowledge gaps due to lack of information and monitoring (Burgman, 2004; McGeoch et al., 2010), inadequate survey details, improper data resolution, undocumented information, data that is not accessible, misidentifications, unresolved uncertainties in nomenclature, and taxa that are not described (Jacobs et al., 2017; Latombe et al., 2017; McGeoch et al., 2012; Mgidi et al., 2007; Pyšek et al., 2008; Regan et al., 2002).

Typically, species lists greatly underestimate the number of introductions (Darling & Carlton, 2018; Genovesi et al., 2013, Richardson et al., 2020), as the number of alien species listed is a result of information availability (McGeoch et al., 2010; Simberloff, 2003). Zengeya et al., (2020a) highlights that numerous listed alien species lack records or studies reporting their occurrence status. Arguably alien species are more likely to be omitted from such lists (as they have not been detected yet) than accidentally included (due to errors in data collection, taxonomic identification, or reporting). Hence, the inclusion of an alien species on a legislated list does not ensure that it is actually present in that country (McGeoch et al., 2012) (e.g. Marlin et al., 2027 & Mayonde et al., 2016).

Despite the field of invasion biology experiencing exponential growth over the past few decades (Richardson et al., 2011), lack of information is still the biggest source of uncertainty when listing IAS, and it frequently leads to an underestimation of the number of IAS in a country, geographic area, or environment (McGeoch et al., 2012). Because of a lack of information, alien species are misidentified or not recognised, their impending threat is not recognised, or the level of risk is incorrectly classified (McGeoch et al., 2012). Considerable efforts have been made to enhance the precision of lists and inventories of alien species (Genovesi et al., 2010, Shine et al., 2010; van Wilgen et al., 2020) but there will always be uncertainty in the process of listing IAS (Burgman, 2004). Yet, by enhancing comprehension of the origin, characteristics, and magnitude of the overall range of uncertainties that affect the process, meaningful efforts can be undertaken to deal with uncertainties where possible (Burgman, 2001, Regan et al., 2002, Walker et al., 2003). This will substantially improve the quality and value of the IAS listing process for policymakers and managers (Butchart et al., 2010).

1.4. South Africa as a case study

The Conservation of Agricultural Resources Act (Act 43 of 1983) instituted the regulation of IAS and consisted of 47 invasive alien plant species that required management and control (Richardson et al., 2020). In 2001, the list was increased to 198 invasive alien plant species (Lukey & Hall, 2020). These alien plant species were also grouped into three different categories: (1) invasive species of no value; (2) recognised invasive species that also have commercial value; and (3) recognised invasive species that have ornamental, but no commercial value (Richardson et al., 2020). In 2014, the Department of Environmental Affairs replaced the Conservation of Agricultural Resources Act by the Alien and Invasive Species regulations published in terms of the National Environmental Management: Biodiversity Act (NEM:BA; Act 10 of 2004) (Kumschick et al., 2020; Wilson, 2023). The regulations were not only replaced but the scope and coverage of IAS were also broadened by including all IAS. The list of taxa that required management and control subsequently expanded to include a total of 559 invasive alien species, with an additional 560 species designated as prohibited for introduction into South Africa (Richardson et al., 2020). These species were split into two broad types of alien species regulatory lists under NEM:BA A&IS Regulations of 2014. The one list consisted of species that are not yet present in the country and can be considered the “prohibited list”, which has since been removed (NEM:BA Alien and Invasive Species Lists, 2020). The second list included species that are in the country and need to be controlled/managed with the aim of reducing their impacts (Kumschick et al., 2020a, NEM:BA Alien and Invasive Species Lists, 2020). The second list groups species into categories based on the benefits and the feasibility of control. These groups have been categorised and named as follows: Category 1a - eradication targets requiring compulsory control or eradication; Category 1b - species with high invasive potential and thus are control targets needing a national management plan; Category 2 - species requiring a permit for restricted activities; Category 3 - species which can remain as long as a permit is obtained for it but eventually need to be assessed and either managed or phased out accordingly (Kumschick et al., 2020).

South Africa has also attempted to address the issue of inaccurate alien species inventories by means of national legislation, as the law mandates that every three years a formal assessment of the state of biological invasions is conducted; and to date two such assessments have been published (van Wilgen & Wilson, 2018; Zengeya & Wilson, 2020). As part of the mandate, the National Environmental Management: Biodiversity Act (Act No. 10 of 2004), which forms the foundation of invasive species management plans and regulation in South Africa, also requires the Minister to create and maintain a national list of invasive alien species known to be present within the country (Zengeya et al., 2020). There are currently several species listed on the alien and invasive species list (NEM:BA A&IS Regulations) that do not appear to be present in the country (Kumschick et al., 2020a). It is thus essential to have evidence-based and transparent standardised procedures for listing alien species (Burgman, 1981; Karasawa & Nakata, 2018; Keller & Springborn, 2014; Schmiedel et al., 2016).

1.5. Research problem

According to Magona et al. (2018), it is ideal for a list of alien species to be based on field observations involving a physical specimen curated in a collection, whose identity is confirmed through both morphological and molecular methods. Most alien species listing decisions are a result of recommendations by panels of scientific experts, often made without specimens(Bertolino et al., 2020; Burgman, 1981; Luque et al., 2014) although it is suggested that ideally, physical specimens should be used for compiling comprehensive species lists (Pyšek et al., 2013). This is often not achieved, and as a result, it may contribute to the errors in alien species lists. According to Wilson et al. (2018), the minimum standards required vary between lists and sometimes a physical specimen is not required at all.

Even though declaring presence is a requirement for inclusion on alien species lists, it is desirable to also record absences (e.g. Latombe et al., 2017). Specifically, before removing taxa from lists, whilst they were known to be present at some point in the past it can be argued that what is required is active evidence of absence. The dependability of regulatory lists is heavily contingent on the methodologies followed during the development processes (Pagad et al., 2022). While the inclusion of an alien species on a regulated list does not imply that it is actually present within a country (McGeoch et al. 2012), it's important to note that species are listed based on their perceived level of threat or potential for establishment and spread. Conversely, omission from a list does not necessarily mean that it doesn't have the potential to become a threat in the future (Luque et al., 2014). Therefore, there has to be a clear procedure for stating that a species is absent.

1.6. Research aims and objectives

This study aims to address the issue of alien species that have been listed as present in the country on regulatory lists, but are absent, or vice versa, by using South Africa as a case study. The primary objective is to develop a protocol for use by any entity tasked with the responsibility of assessing the presence or absence of a specific alien species within a country. The study tested the protocol using two case study species: *Calluna vulgaris* and *Euphorbia esula*. These species are categorised as listed under the National Environmental Management: Biodiversity Act (Act No. 10 of 2004): Alien and Invasive Species Regulations. Although they have been reported as present within the country based on anecdotal evidence or observations, their presence has not been formally recorded in scientific literature or official records and confirmed.

The following steps were identified and used to address the dissertation objectives:

- Identify instances where species have been recorded in South Africa, but their presence is disputed;
- Develop a protocol so alien species can potentially be classified as absent despite previous evidence of presence; and
- Apply the protocol to case studies in South Africa.

1.7. Dissertation outline

Chapter two of this dissertation investigates selected taxa listed under the National Environmental Management: Biodiversity Act (Act No. 10 of 2004): Alien and Invasive Species Regulations. It centres on those taxa whose presence is disputed and scrutinises the intricacies of the uncertainty surrounding their recorded status. Furthermore, it introduces a protocol designed to guide various entities in resolving whether an alien species is absent or present when dealing with conflicting or low-quality information. Chapter three focuses on the application of this protocol to two taxa whose presence has been disputed. Chapter four summarises the findings of the dissertation.

This dissertation follows a structured approach, with Chapters two and three intended for publication as standalone papers. Consequently, there may be instances of methodological and contextual overlap between the chapters.

Chapter Two: A proposed protocol to classify an alien species as absent despite previous evidence of presence: South Africa as a case study.

2.1. Abstract

Reliable listings of alien species are necessary for effectively managing biological invasions. When an invasive species is confirmed to be absent, it enables conservation efforts to be directed towards addressing those alien species that are present. However, declaring an alien species as absent cannot be done with perfect certainty (it is fundamentally impossible to prove an absence), and if an alien species is incorrectly declared as absent, the opportunity for eradication or significantly reducing spread may be lost. To address this challenge, a protocol was developed to classify the presence of an alien species in a country. This protocol relies on assessing historical data to determine the probability of the species being present in the past and evaluates the likelihood that it has persisted until the present time. The assessment requires data regarding temporal and spatial availability of evidence indicating the species' presence, the probability that populations have remained extant since the most recent observation, the thoroughness and rigor of search efforts to detect any extant individuals, the certainty and confidence in species identification, and the quality of the samples or evidence collected. The proposed protocol is based on literature reviews, argument maps, and equations. To illustrate the approach, the study uses the example of the Chilean black urchin [*Tetrapygus niger* (Molina, 1782)] in South Africa, a taxon that is regulated as an invasive species in the country but that has not been observed in over a decade. By presenting arguments both for and against the presence of *T. niger*, it is argued that the taxon is probably no longer present in South Africa, and the recommendation is that *T. niger* is removed from the regulatory lists. The proposed protocol's simplicity and flexibility thus make it a valuable tool, particularly in data-limited situations, complementing more complex methods when sufficient data are available. It also facilitates evidence-informed and transparent decision-making.

2.2. Introduction

It is important for a country to have accurate lists of alien species (Latombe et al., 2017). These lists help to advise and guide species management prioritisation, promote the implementation of management plans, report on biodiversity targets, raise public awareness, determine research priorities (Rocha et al., 2013), and identify the state and trends of biological invasions in order to inform policy (European Commission, 2014; Hulme et al., 2009; Kolar & Lodge, 2001; Ricciardi et al., 2000; Shine et al., 2005; Simberloff et al., 2005; Wittenberg and Cock, 2005) as governments and managing authorities make laws and policies based on alien species lists (Shine et al., 2005; Lodge et al., 2006). Furthermore, these lists can assist with the improvement of the monitoring of alien species and the prevention of further introductions of alien species (Burgiel et al., 2006; García-de-Lomas & Vilà, 2015; Pagad et al., 2015). Even though alien species lists will never be complete or completely accurate, an inadequate or poorly contextualised list of alien species can hinder effective management, prevention and control measures (Kolar & Lodge, 2001; Pyscaron, 2003; McGeoch et al., 2012). Therefore, it is essential that the information in these inventories be as thorough and detailed as possible (McNeely et al., 2005; Meyerson & Mooney, 2007; Stoett, 2010). A standard, systematic, and evidence-based approach to compiling and maintaining inventories of alien species can significantly increase their usefulness (McGeoch et al., 2012). When alien species are proposed to be prioritised for management, current and accurate data is vital (Groom et al., 2015). The success of the processes involved in listing alien species depends largely on the scientific evidence available (Simberloff, 2003). Expert input is often used in the listing of alien species to fill in known knowledge gaps, compile and review data, and make final listing decisions (Burgman, 2004; Cook et al., 2010; Hulme et al., 2009; Hulme et al., 2009a). Expert judgment is most commonly utilised to make choices on listing in largely unexplored geographic areas, for species that are not well known (McGeoch et al., 2012), and/or when obtaining reliable data is difficult or impossible (Burgman, 2004). Scientific experts are expected to make trustworthy, transparent, and consistent decisions on behalf of the public; however, expert judgments are prone to inaccuracies and errors (Burgman, 2001, 2004).

When assessing the presence or absence of alien species, two major categories of information should be considered: 1) the nature and timing of records of presence (e.g. Sightings, reports, and specimen collections); and 2) the timing, scope, and the suitability of surveys and searches. A straightforward sampling method commonly employed to estimate occupancy of a species within a particular habitat requires only information about the number of visits made by field researchers to each location and the duration of time spent during those visits. These visits and time spent represent the search efforts aimed at either locating the target species directly or identifying signs suggesting the species' presence (MacKensie et al., 2018). The intensity of the search effort may also have an influence on the likelihood of finding an incipient population (Hauser et al., 2016; Yackel Adams et al., 2018). The outcome of a survey considering these variables is a list of species that are 'present' (species detected) and 'absent' (species not detected) (MacKensie et al., 2018). These count-based inferences can however be flawed, and as a result, species may be considered absent whilst they are present (MacKensie et al., 2018) and vice versa. This is particularly problematic if detection is difficult. For example, some alien species can take a while to establish (called a "lag phase"), so they might not be detected immediately after being introduced (Crooks, 2005; Moodley et al., 2014; van Wilgen et al., 2020). Additionally, small populations are also harder to find and more often lead to false absences (Yackel Adams et al., 2021). When attempting to detect, identify, and confirm the presence or absence of such alien species within a country, extensive monitoring is required because it may have been overlooked during initial surveys (Cohen et al., 2002; Kery, 2002; Guillera-Arroita et al., 2014). In many cases, determining whether a species is absent or present, but in small numbers and/or at a very low density, can thus be challenging (Kery, 2002).

Determining whether a species is absent from a country or has been overlooked is essential. If a species' presence has been disregarded in this manner, it might not receive the necessary intervention or proper management. As a result, the species could establish and become invasive, leading to a missed opportunity for eradication (Goslan et al., 2010) (e.g. Spear, 2018; Matthys et al., 2022; Mack & Lonsdale., 2002. According to the IUCN (2012) a species is considered extinct (absent) when there is no reasonable doubt that the last individual has died and exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), throughout its historic range have failed to record an individual. A species is also considered vanished (absent) when they have only been reported once or a few times in the past and have not been seen in a very long time; for the vast majority of vanished species, this means in the previous 25 years (Pyšek et al., 2012). This is particularly challenging due to certain life histories that allow for long persistence times, reducing detectability (Figueiredo et al. 2019). For example, in some cases, plant taxa can persist at very low densities, remaining functionally but not actually extinct and others may only be documented in a single collection with vague locality details, making it difficult to ascertain their extant status (Gilbert & Levine, 2013). Factors that complicate extinction assessments include challenges in detecting inconspicuous and cryptic taxa, along with limited information about their ecology (Downey & Richardson, 2016; Gray, 2019).

Therefore, while finding a specimen may demonstrate the presence of a species, concluding with absolute confidence that it is absent is impossible. Only probabilistic statements/claims may be made about the likelihood of its absence (Kery, 2002). Several approaches and models have been developed to incorporate error estimates into the assumption of a species' absence. However, these methods are typically based on only one factor each when estimating the likelihood of a species' absence. For example, they may consider quality of surveys (IUCN, 2001), trends in sighting intervals (Jarić & Ebenhard, 2010), attributes of the target species (Kim et al., 2020)certainty of sighting records (Lee et al., 2014, Rout et al., 2009) or number of visits to the reported location (Reed, 1996). In essence, these methods do not consider multiple factors simultaneously but instead concentrate on a single factor. A more suitable approach would be to consider all these factor as well as factors that are evenly applicable across taxa. So rather than focusing simply on the unique factors of a single species or taxon, it is preferable to include factors that are relevant across other types of organisms. As a result, the evaluation will be more thorough and balanced, since it includes both the specific characteristics of each taxon as well as the common factors that determine their presence or absence. This method enables a more comprehensive and complete assessment, enabling greater accuracy and dependability in determining the presence or absence of species.

In many countries there is a lack of certainty regarding the presence, past presence, or current presence of some alien taxa; and there is currently no systematic way to address this issue. As a result, the purpose of this study was to develop a structured protocol to determine the probability that a species is absent (or present). The proposed protocol seeks to provide decision-makers with a transparent and consistent process to make accurate declarations of alien species' absence, and in doing so ensure appropriate intervention and management strategies are in place if it is not absent.

This chapter has two specific objectives:

- To identify species that are currently categorised as alien in South Africa but may have been incorrectly assumed to be present, or their presence remains uncertain and disputed;
- To create a transparent and consistent protocol to classify an alien species as absent from South Africa despite previous evidence of presence.

2.3. Research design and methodology

This study includes quantitative (creating a species list) and qualitative data (constructing a protocol) to achieve the above aims.

2.3.1. Assessing the status of listed alien species

i. Data collection

To assess the status of listed alien species in South Africa, a compilation was made from the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004): Alien and Invasive Species List 2020 (Department of Environment, Forestry, and Fisheries, 2020). This compilation comprises species assumed to be absent from the country due to uncertainties. This was done in a systematic way by running a search on the Global Biodiversity Information Facility (GBIF) (www.data.gbif.org), an online spotter network (https://www.iNaturalist.org), GenBank (https://www.ncbi.nlm.nih.gov/genbank/), the database of herbarium records (https://www.newposa.sanbi.org), the Web of Science (https://www.webofscience.com), and on Google Scholar using the species name and “South Africa" for all listed species to see which have physical evidence or historical records of being present in the country. Taxa with substantial, robust, and reliable supporting data indicating their presence in the country at the present time as well as taxa whose presence has been validated or verified through thorough assessment or observation, were not considered. Only species on the NEM:BA A&IS Regulations List of Alien and Invasive Species (2020) with disputed presence data (such as old records, poor quality records, etc.) were considered. The following information was considered for each record: the regulatory grouping of the species (i.e. legal categorisation, such as being classified as invasive or prohibited; for example, a species may be categorised as "invasive" under South African regulations); the accepted scientific name according to Plants of the World Online (https://powo.science.kew.org/) (for plants) and Global Biodiversity Information Facility (https://www.gbif.org/) (for animals); the listing category of the species according to NEM:BA A&IS Regulations; the type of record; the date of the most recent occurrence record; and references to the occurrence data/record.

ii. Data categorisation

The obtained data was categorised based on the terms outlined by the Darwin Core List, following the criteria specified by the category definitions. The Darwin Core standard was used to do so (Darwin Core Task Group, 2009). The standard consists of a glossary of terminology designed to enable the exchange of biological diversity information by giving IDs, labels, and definitions. The categorisation involved organising the data based on these predefined elements and terms provided by the standard. Each piece of information collected was matched to the corresponding category within the Darwin Core glossary. For example, data related to taxa, occurrences, and other relevant information were assigned to their respective categories within the standard. This process ensured that the data were structured and organised according to the established framework of the Darwin Core, allowing for consistency and compatibility in biodiversity information exchange.

2.3.2. A workshop to develop the themes for a protocol

An online workshop was held with researchers and practitioners working on biological invasions to construct the themes for a protocol to classify alien species as absent or present from a country. Participants came from environmental, higher education and conservation institutions. Workshop participation was optional and anonymous. By attending online, participants consented to the use of their input. Participants were also informed that they may withdraw from the research, reject consent, and refuse to participate (Ethics Approval Reference no: 214284123/11/2020).

The workshop's four main topics were: 1) historical sources or proof of a species' presence in a country; 2) search efforts to determine species presence/absence; 3) probable grounds for absence; and 4) repercussions of "wrong" statements. To obtain the output, the host gave a formal presentation followed by participant discussions. The host informed attendees on the workshop format and desired objectives, divided them into four breakaway groups which then discussed a topic until reaching agreement. After returning to the main group, a representative from each group presented the outcomes of their discussions with all participants, and the presenter finished the workshop with an overview of key points. The workshop recordings including the main group and breakaway groups' discussions ensured the feedback was appropriately captured. From the workshop, the most relevant themes were identified by analysing the discussions.

2.3.3. Developing the protocol for declaring a species as absent

Based on the identified themes from the workshop, a literature review of the species under assessment was conducted to gather relevant information and establish a comprehensive understanding. Additionally, an argument map was created to visualise the evidence presented in the literature review, facilitating a structured analysis of the available data. To enhance the protocol's robustness, a probability scoring guide was integrated into the argument map, allowing for a systematic evaluation of the evidence and uncertainties surrounding the species' presence or absence. While the protocol aims to provide a structured framework for decision-making, it is important to acknowledge the existence of uncertainties inherent in the assessment process (e.g. McGeoch et al. 2012). Some uncertainties, such as those related to the quality and completeness of available data, can be minimised through thorough information gathering, including the literature review. However, it is recognised that certain uncertainties may persist despite efforts to mitigate them. In such cases, probability estimates are included as part of the protocol to provide guidance for decision-making, allowing for informed judgments to be made based on the available evidence.

2.4. Results

2.4.1. Assessing the status of listed alien species

Out of the 560 taxa regulated under the NEM:BA A&IS Lists of 2020 (Wilson, 2023), 11 taxa were found to have disputed presence data (Table 2.1). There were various reasons for disputed presence data. Seven of the taxa only had preserved specimen records, two were only human observations, and another two were only material citations without additional observational or interpretive data. Eleven of the 12 had only one record of presence in South Africa. These taxa must have a record/that represent historical occurrence in the country in order to be included, however the confidence level of current presence may be low due to a lack of supporting evidence. Additionally, two of the taxa had records with no dates, seven were last recorded ten or more years ago and two were recorded seven and five years ago. Therefore, if records of a taxa are not dated, are old and without recent confirmation, there is reason to dispute their presence.

Table 2.1: A list of alien species regulated in South Africa that are recorded as present but may be absent or whose presence is disputed based on the quality, quantity and age of records (i.e. one record, old records, poor quality records, whether permits were issued, etc.). The nomenclature and status as regulated is based on the NEMBA A&IS Lists of 2020 (the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004)'s Alien & Invasive Species Regulations of 2022 (Department of Environment, Forestry, and Fisheries, 2020). For a taxon listed as Category 2 a permit may be issued in respect of South Africa's National Environmental Management: Biodiversity Act's Alien and Invasive Species Regulations over the period October 2014–December 2020 to keep the taxon and if permits were issued, this is taken as evidence that the taxon is in the country (albeit with low confidence) (SANBI, 2023; SANBI & CIB, 2020).

scientificName	**eventDate**	**basisOfRecord**	**associatedReferences**	**Reason for dispute of presence**
Albisia procera (Roxb.) Benth.	1957	PreservedSpecimen	Ranwashe, 2022	One record. The record was a preserved specimen.
Anolis carolinensis Voigt, 1832	1947	PreservedSpecimen	Dondorp & Creuwels, 2022.	Published without coordinates. First and only record.
Euphorbia esula L.	2006	MaterialCitation	Henderson & Wilson, 2017	One record in SAPIA. No photographic evidence or live specimen. Searched for and not found (chapter 3 of this dissertation).
Gehyra mutilata (Wiegmann, 1834)	1869	PreservedSpecimen	Finnish Biodiversity Information Facility, 2022.	One record. Published without coordinates. No photographic or live specimen.
Hypericum androsaemum L.	2015	HumanObservation	Mary-Hunter, 2015.	One informal record. Record published seven years ago. No confirmed records of presence in South Africa. Listed on NEM:BA for precautionary reasons; invasive and restricted in Australia.
Kobus vardonii (Livingstone, 1857)	None	PreservedSpecimen	Trombone, 2016.	Only one record. Record published without coordinates or event date. No photographic or live specimen. No permits issued.
Macaca fascicularis (Raffles, 1821)	None	PreservedSpecimen	Williams, 2011.	Two records, however both were published without coordinates or event date. No photographic or live specimen.
Rhus glabra L.	1983	PreservedSpecimen	Marais, 2019; Shah & Wikström, 2022.	One record only. Published without coordinates. Second record published without date or without coordinates.
Schefflera elegantissima (H.J.Veitch ex Mast.) Lowry & Frodin	2018	HumanObservation	Vos, 2018.	One record of presence in South Africa. Only record was in a garden. Record published four years ago.
Tetrapygus niger (Molina, 1782)	2007	MaterialCitation	Mabin et al. 2015	Record of eradication attempt of only known population. No other record with physical evidence available.
Trogoderma granarium Everts, 1898	1954	MaterialCitation, PreservedSpecimen	van Noort & Ranwashe, 2020; Viljoen, 1990.	One confirmed record. Reported to be eradicated.

2.4.2. Workshop outcomes

The workshop was attended by 33 participants and lasted for an hour. A summary and description of the data (i.e. themes) that emerged are described below:

Theme one: One cannot state explicitly whether a species is absent but rather estimate the probability of absence, along with a measurement of confidence of the probability. The recommended metric to assist with management of alien species is to indicate the probability of absence along with a measurement of confidence in this the probability (e.g. Kery, 2002; Russell et al., 2017; Yackel Adams et al., 2018) but this is typically based on the quantity, quality and reliability of data on the taxon that is available and accessible. The adequacy of documentation and data varies widely among taxa, leading to discrepancies in the quality and quantity of information accessible for assessment. This inequality in documentation and data availability poses a challenge when determining the probability of absence and the associated confidence level, as the reliability of these assessments relies heavily on the completeness and accuracy of available information. Therefore, efforts to improve documentation and data collection for various taxa are crucial for enhancing the accuracy and reliability of assessments regarding species absence.

Theme two: Various aspects of search effort need to be considered (e.g. the level of expertise of those searching for a species, how many species were searched for, how many times it was searched for, and whether it is part of a formal or informal search effort). The results from the search effort will support the probability that the species is no longer present. Information to guide the measuring/scoring of confidence in the probability will be required to support decision-making on determining search effort.

Theme three: Information on the certainty of identification (I.e., who identified it) and the quality of samples or evidence of the initial reporting needs to be considered in order to determine the probability that the species has been present in the country.

Theme four: The probability that a species is no longer present is influenced by species characteristics, such as dispersal ability, and the consideration of the number of individuals/size of population recorded.

Theme five: A variety of factors, including fires, previous habitat modification, propagule pressure, herbivore release, human use, pollinators, and habitat compatibility, determine the invasion success (Geerts & Adedoja, 2021; Geerts et al., 2013; Geerts et al., 2016, 2017; Honig et al., 1992; Mangachena & Geerts, 2019; Sundaram et al., 2015; Wansell et al., 2022) and their likelihood needed to be considered. The likelihood that a species will successfully invade (invasive potential) after introduction may inform us on the probability that all populations may be lost after introduction. The protocol should consider the characteristics and traits of the species being studied to support why the species may or may not be absent.

Theme six: Expert opinion, experience and observation which refer to the insights, knowledge, and observations provided by individuals who possess expertise in relevant fields. These should be considered within the protocol. It should however be collated in a structured way, with explicit listing of limitations, assumptions, and information gaps.

2.4.3. The proposed protocol

i. Determining the probability that a species is absent from a country

Equation 1

$$P(absence) = 1 - P(it\ was\ present) \times P(it\ is\ still\ present)$$

Equation 1 calculates the probability of the species being absent in a particular area. It does this by considering the likelihood that the species was present and is still present.

Equation 2

$$P(it\ was\ present) = F(certainty\ of\ identification) \times F(quality\ of\ samples/evidence)$$

Equation 2 calculates the probability of a species' initial presence in an area by considering two interrelated factors: the certainty of species identification and the quality of supporting evidence or samples. The certainty of identification is influenced and determined by the quality of supporting evidence or samples. This factor assesses how sure the assessor is that the identified species is the correct one, taking into account expert identification and reliable methods. Simultaneously, the quality of samples/evidence evaluates the reliability of supporting data, including photographic records and specimens. These intertwined factors are multiplied together to estimate the overall likelihood of the species being present initially.

Equation 3

$$P(it\ is\ still\ present) = F(likelihood\ populations\ have\ been\ lost)$$

Estimation of *P(it is still present)* is determined by two key factors: how recently evidence of its presence was found and the thoroughness of search efforts without it being found. Together these factors contribute to the likelihood populations have been lost. A higher score for each factor implies a greater likelihood of absence, while lower scores suggest a higher probability of current or recent presence (Equation 3).

The determination of *P(it is still present)* and *P(it was present)* depends on qualitative information assessed through expert judgment. To reduce subjective bias, inconsistencies, vagueness, expert fatigue, and other controllable sources of uncertainty in such situations, a structured approach is proposed. Employing an argument map is one method to organise and present the reasoning behind the probability of absence estimate, using qualitative information as the foundation for the analysis (Keith et al., 2017). In order to obtain the relevant information for the argument map, an extensive literature review should be conducted on the case study species.

ii. Literature review of case study species

For each case study species, occurrence data for the area should be obtained. Occurrence data included all information relevant to the equation, which calculates the probability of the species' absence in a particular area such as historical presence records, evidence of current presence or absence, and any other relevant data points. References used should be provided to ensure transparency and traceability in the analysis. Information gathered from the literature review may include, but is not limited to: certainty of species identification; the quality of reported samples or evidence of the species in the country, how long ago most recent evidence of presence was reported, events and species characteristics that may affect the likelihood that all populations have been lost since the last report, and search efforts for the species since it was reported. The results of the literature review should then be consulted and included in the next step of the protocol, which is an argument map.

iii. Argument map

Following a similar approach as Van der Colff et al. (2023), this study uses an adapted argument map approach derived from Walton (2013) and Keith et al. (2017) to enhance the consistency and transparency of absence assessments, as well as to handle uncertainties. The argument map is based on a probabilistic statement to determine the likelihood that the species being considered is absent after being reported and assumed to be present. The proposition that a species is absent (claim) is evaluated by using biological understanding to outline all the credible reasons (evidence) why the proposition could be true (reasons) or false (objections). An argument map is a logical way to organise these ideas since it is based on qualitative information and evidence that supports an estimate. Argument maps are centred on a claim, such as 'the species is absent', and the claim is then supported by reasons, evidence, and sources (Okada et al., 2008). When supporting the claim in this way, counterfactuals or lines of reasoning will arise that naturally lead to and support the opposite claim (I.e. the species is present). This is represented in the argument map as 'objections'. Each 'objection' is linked to the 'reasons' or other portions of evidence. All the evidence included in the argument map, and their weight, need to be considered by users of the protocol to form an objective opinion on the probability that the species is absent. Ideally, each branch of evidence depicted in the argument map should be substantiated by a corresponding source. Published data and studies, as well as ecological data, personal observations, location records, scientific literature and informal observations and expert opinion, may be used as sources.

iv. Probability scoring

There is no formal means to assign weight or a score of confidence in a given probability that the species is absent. Rather a general guide is proposed to assign values for scoring probability for the equations (see Appendix 2.1 for a more detailed guideline). Considerations when weighing up the probability scoring of each section of the equation is discussed below:

Equation 1: P(absence)

Allocating an overall high absence score hinges on having comprehensive knowledge of the taxon's biology, its interactions with the introduced environment, and its range (Van der Colff et al, 2023). However, a significant limitation is its reliance on detailed knowledge about the taxa under assessment. In many instances, taxa are only identified by their type locality, accompanied by limited biological data, which necessitates assumptions based on closely related taxa. As a result, they may receive lower absence probability scores with significant uncertainty.

The decision on the degree of certainty will represent a trade-off between the risks associated with the two options: (1) declaring the taxon absent and allowing a population to grow and spread uncontrolled, reducing opportunities for proactive control; and (2) declaring that the species is present and wasting resources and effort in monitoring.

Equation 2: P(it was present)

F(certainty of identification)

A higher scoring is justified when there is substantial evidence supporting the identification, including multiple confirmations, detailed information, expertise of the reporting party (Burgman, 1981), and recognition from reputable sources. A lower scoring is appropriate when there are uncertainties or shortcomings in the identification process, such as limited evidence, lack of expertise, presence of similar-looking species, or lack of verification (Magona et al., 2018).

F(quality of samples or evidence)

A higher score is justified when the evidence supporting the absence claim is strong, well-documented, and sourced from reputable sources (Kery, 2002). Factors justifying a higher score include comprehensive surveys conducted by experts, reproducibility by independent researchers, evidence from reputable scientific journals or governmental reports, multiple data points supporting the claim, data transparency, and expert input. A lower score is justified when the available evidence is limited, lacks detail, or suffers from methodological flaws that introduce uncertainty or bias. Factors justifying a lower score include evidence based on a single or limited number of reports, incomplete records lacking critical information, ambiguity in the evidence, lack of expert review, and conflicting reports suggesting the species may still be present.

Equation 3: P(it is still present)

F(how long-ago evidence of presence was)

The confirmation of a species' current presence is time dependent. Time since last recorded also known as residence time is therefore important to determine whether a species may or may not still be present. The longer the time since the last record, the higher the likelihood that a taxon is not still present.

F(likelihood populations have not been lost)

This scoring aims to estimate the probability of complete population loss for a species within a specific region. This assessment considers various factors, including historical trends, habitat suitability, and recent evidence. A higher score suggests a greater likelihood of all populations being lost, typically due to a combination of adverse historical trends, unsuitable habitat, and a lack of recent sightings or evidence. Conversely, a lower score implies a lower probability of complete population loss, leaving room for the possibility that the species may still persist, though potentially in limited numbers or isolated locations. Additionally, the scoring accounts for the likelihood of search efforts, even when people don't expect to find the species, which can influence the assessment of population loss likelihood.

F(search effort)

This involves evaluating the extent and rigor of search efforts conducted to detect the presence of a species, significantly impacting the confidence in the assessment. A higher score is assigned when comprehensive, well-organised, and persistent search strategies have been employed, increasing the likelihood of detecting the species if it is present. Conversely, a lower score is warranted when search efforts have been limited, are sporadic, or inadequately conducted, reducing the chances of confirming the species' presence. The thoroughness of search efforts directly influences the reliability of the absence estimation, contributing to the overall confidence in the assessment.

2.4.4. Protocol explained: The Chilean black urchin (*Tetraphygus niger*) (Molina, 1782) as a case study

The examination of the Chilean black urchin (*Tetraphygus niger*) (Molina, 1782), an alien species on the NEM:BA 2020 Alien and Invasive Species Lists of South Africa, was conducted to assess the applicability of the proposed protocol.

i. Step 1: Background literature review

Tetrapygus niger is native to the Pacific coast of South America, from the north of Peru to the south of Chile (Clark, 1910). It is found in rocky habitats that range from the intertidal zone to 40 m depth on rocky substrata or artificial structures (Dumont et al., 2011), especially in regions with dense kelp forests and powerful wave surges. Juveniles are quite cryptic (Rodrígues & Ojeda, 1993) however, adults are not cryptic as they are readily distinguishable and identifiable (Schults, 2006). In 2007 alien populations were found in two farmed aquaculture dams in Alexander Bay, a town on the west coast of South Africa (Haupt et al., 2010; Mabin et al., 2015). *Tetrapygus niger* larvae were thought to have been introduced with the spat from the Pacific oyster (*Crassostrea gigas*) (Mabin et al., 2015). As part of routine husbandry procedures, equipment was rinsed, and the runoff was discharged down a dune and into the intertidal zone, raising the possibility that the urchin may have spread to the nearby shoreline (Mabin et al., 2015). This is especially concerning because the South African west coast offers a very similar habitat to the urchin's native range (Bustamante & Branch, 1996; Rodrígues, 2003; Rodrígues & Ojeda, 1993; Wieters et al., 2009). In September 2014, the two aquaculture dams from which it was previously reported, as well as the intertidal and subtidal areas surrounding the dams' discharge sites were surveyed thoroughly (Mabin et al., 2015). One of the dams had dried up due to the oyster farms having been abandoned since the last survey in 2007. However, a new dam was developed beside the second dam (Mabin et al., 2015). None of the habitats that were examined in 2014 supported living *T. niger*; only shells were found in the one empty dam. The two other dams did not support urchin species as they were hypersaline (Mabin et al., 2015). Mabin et al. (2015) suggest that there is no evidence that the species naturalised outside of captivity, and it doesn't seem to have spread from these dams. According to Mabin et al. (2015), *Tetrapygus niger* is also unlikely to be reintroduced to Alexander Bay because the aquaculture facility is no longer being used for aquaculture. However, it could potentially be introduced into other oyster farming facilities in South Africa. This indicates that there are still viable vectors and pathways of introduction for this species in South Africa. *Tetrapygus niger* is currently listed as a Category 1a species (target for eradication) under NEM:BA A&I Regulations but is not included in the World Register of Introduced Marine Species (WRIMS) (Pagad et al., 2015). Mabin et al. (2015) recommend that this record be classified as "absent" if it is added to the WRIMS and that it is appropriate to keep the species on a prohibited and/or watch list until there is evidence of a lack of vectors and pathways in South Africa.

ii. *Step 2 & 3: Argument map and scoring the equation*

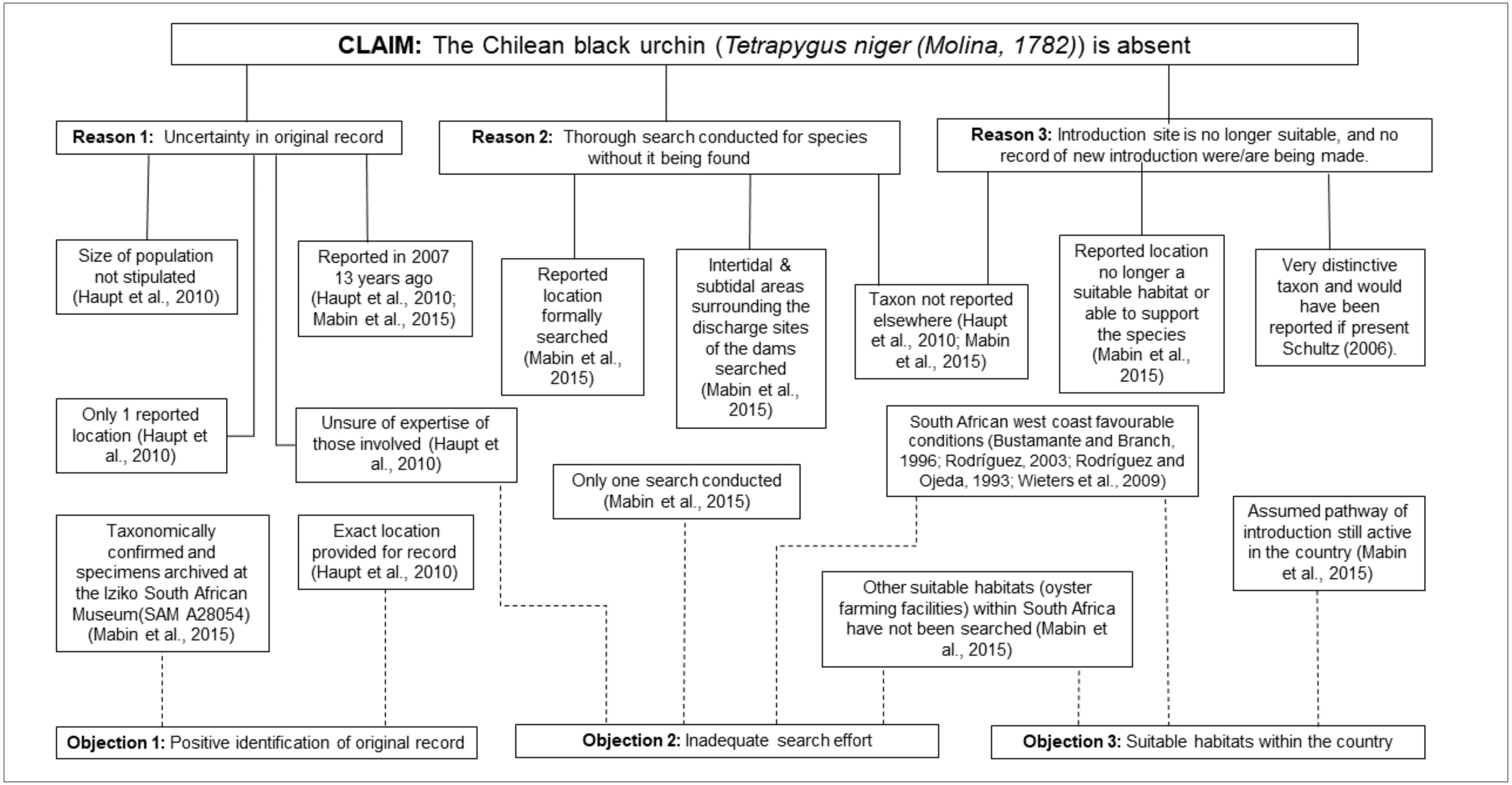


Figure 2.1: Argument map for the claim that the Chilean black urchin [Tetrapygus niger (Molina, 1782)] is absent from South Africa. The claim appears in the uppermost box titled 'claim' and is based on various reasons, shown in boxes titled 'reason' and each reason is supported by evidence. The argument map may include sources supporting each piece of evidence or may be omitted for simplicity (as was done here). 'Objection' to the evidence and by inference, the reasons, are shown in boxes titled 'Objection'.

The ‘claim' in Figure 2.1 states that “The Chilean black urchin (*Tetrapygus niger,* (Molina, 1782)) is absent". To formulate ‘reasons’ to support the ‘claim’, ‘evidence’ was collected from the literature. The proposal involves establishing a confidence level to substantiate the ‘claim’.

Equation 2: *P(it was present)*

F(Certainty of identification) and F(Quality of evidence/sample)

‘Reason 1’ states that there may be uncertainty in the original record. This speaks to the *Certainty of identification* of *T. niger* and the *Quality of the evidence/samples*. The evidence supporting this reason includes the absence of information about the size of the population at the observed location and the fact that there is only one reported location for the species. Furthermore, the initial report was made in 2007, which may raise concerns about the accuracy of the identification because it was not reported again after that. On the other hand, ‘Objection 1’ opposes this uncertainty, stating that there is a positive identification of the original record. This objection is supported by concrete evidence, including taxonomic confirmation of the species and the archiving of specimens at the Iziko South African Museum. Additionally, the provision of the exact location of the original record adds further reliability to the identification. However, while ‘Reason 1’ raises valid concerns under uncertainty, it might not fully outweigh ‘Objection 1’ as there is substantial evidence to support the certainty of identification. For both *Certainty of identification* and the *Quality of the evidence/samples* a high score may be allocated.

This means there's a high likelihood that the species is absent from the country.

Equation 3: *P(it has not disappeared)*

P(Likelihood of all populations being lost)

How long ago evidence of presence was for the species' presence is a point of consideration under ‘Reason 1’ raises concerns about the 13-year-old evidence, the lack of details on population size, and the expertise of those involved, introducing uncertainties. However, ‘Objection 1’ holds equal weight, providing substantial evidence that the species was archived more recently, which counter these uncertainties and boost confidence in the species' presence. ‘Reason 1’ and ‘Objection 1’ carries equal weight, suggesting a medium score. ‘Reason 2’ emphasises thorough searches in the reported location, but ‘Objection 2’ questions the expertise of those involved and points out unexplored areas with potential suitable habitats. ‘Reason 2’ and ‘Objection 2’ carries equal weight, raising concerns about the overall thoroughness of the search, resulting in a medium score. ‘Reason 3’ suggests potential population loss due to an unsuitable original introduction site and a lack of new introductions. However, ‘Objection 3 ‘carries more weight, highlighting the presence of unsearched suitable habitats and an active assumed pathway for introduction in the country.

‘Objection 3’ counters ‘Reason 3’, indicating potential alternative habitats, and carries more weight, leading to a high score.

Equation 1: *P(absence)*

In this case, the thoroughness and rigor of the search efforts without it being found after the eradication attempt will heavily influence the overall probability of absence. When combining the factors included in equation 2 and 3, for Equation 1 will be allocated an overall high probability of absence.

iii. Step 4: Implications of the evaluation

The evaluation indicates that there is a substantial probability of the Chilean black urchin (*Tetrapygus niger*) being absent for South Africa supporting Mabin et al. (2015)’s recommendations. However, it will be necessary to conduct additional species-specific surveys at oyster farms and engage directly with oyster farmers to confirm there are no other populations in South Africa.

2.5. Discussion and recommendations

One of the key benefits of this study is the facilitation of cross-taxa analyses. By establishing a structured method for estimating the probability of species absence, the protocol offers a standardised approach that can be applied to various taxa. Stakeholders may engage in more effective and transparent dialogues by using the protocol, resulting in better-informed choices and actions. The protocol appears to be structured and systematic, integrating various elements like literature review, argument mapping, and scoring equations. It provides a comprehensive approach to classify the presence or absence of an alien species, demonstrated through the case of the Chilean black urchin. The use of the case study demonstrates the practical applicability of the protocol. It showcases its flexibility and suitability for data-limited situations. The protocol considers objections and reasons, providing a balanced evaluation. For instance, objections related to recent archival evidence counter concerns about the age of evidence, adding nuance to the assessment. In other words, new information found in historical records or documents contradicts concerns about the age of evidence, thereby adding complexity or detail to the assessment

The application of argument maps also offers as a valuable tool in pinpointing taxa suspected to be absent from a country. This method helps identify taxa warranting further investigation through field searches to assess potential conservation intervention (Van der Colff et al, 2023). An argument map offers a reasonably comprehensive and practical approach to integrate evidence of different types, enabling the generation of an overall estimate of a taxon's presence. This method, along with structured gathering of information, is anticipated to significantly enhance the consistency and accuracy of expert opinions on absences, as experts are compelled to provide explicit insights into factors influencing absence likelihood (Kieth et al., 2017). Moreover, this approach facilitates discussions about uncertainty, settling opposing opinions by using specific probabilities. One of the advantages of this approach is the ability to quantify the uncertainty surrounding the estimate, arising from underlying observational errors. The equation is also a valid way to calculate the probability of absence while considering the importance of different factors and their complementary probabilities. It allows for a flexible and customisable approach to modelling the likelihood of absence based on the specified factors and weights. To refine the assessment's precision, it is proposed to assign weights to factors based on their perceived influence on uncertainty. For instance, giving higher weight to search efforts over the age of evidence if it is deemed to have a greater impact. This approach would introduce a more nuanced and quantitative dimension to the evaluation process.

The protocol currently assesses factors qualitatively, acknowledging uncertainties without specifying their range or interval. To enhance precision, it is recommended that each factor incorporates a quantifiable range or interval, indicating the extent of uncertainty. Expert input or statistical methods could be employed to establish these ranges, offering a more nuanced and quantifiable approach to uncertainty assessment. It is recommended that assessors seek guidance from experts when applying this protocol to address variations among assessors. It is advised that when seeking out experts and working with them, the assessor/s must do the following: determine what knowledge and skills are needed to solve the problem at hand, choose the experts, obtain information, evaluate the reliability of the information, put together data from different experts, and then use the protocol to make decisions (Burgman, 2004). Additionally, given that the interpretation of data by different experts can vary based on their individual biases, levels of uncertainty, and access to distinct data sources, their assessments of uncertainty levels may conflict or differ. To address this issue and promote a more comprehensive and dependable assessment, it is recommended to use multi-author approach or involve a range of experts the consultation process when utilising the protocol. By engaging multiple experts can harness their collective knowledge and diverse perspectives, leading to a more robust and well-rounded evaluation. The incorporation of expertise from various specialists enables the synthesis of comprehensive insights and facilitates the attainment of well-informed conclusions. Furthermore, Burgman's (2004) suggests ways to improve the reliability and transparency of expert contributions in listing activities, providing practical guidance to mitigate potential expert bias and ensure a thorough assessment process within the protocol. The protocol's use of argument maps also offers an objective way to address subjective expert opinions, and these maps can be adapted as new information emerges, reducing uncertainties over time and prompting updates to assessments for specific species in particular areas.

Moreover, the identification and monitoring of potential pathways of introduction become key components of the process. Identifying the pathways through which the species might be introduced allows for a comprehensive understanding of the potential risks. Implementing a responsive systems is essential to facilitate early detection in case of any new incursions. These systems should be designed to swiftly identify the presence of the species within the targeted areas. By establishing efficient detection mechanisms, the authorities can take immediate action, whether it be eradication or other appropriate measures, to prevent the species from becoming established or spreading further.

Declaring a species as absent requires a multi-faceted approach, involving specialised surveys, engagement with relevant stakeholders, identification and monitoring of potential introduction pathways, and the establishment of efficient detection systems. These measures are necessary to ensure the accuracy of the declaration and to proactively manage and respond to any potential threats posed by the case study species.

2.6. Conclusion

This study conducted a thorough analysis of essential criteria for declaring a species absent from a country, offering valuable insights and recommendations to enhance the assessment process. The protocol's balanced evaluation, considering objections and reasons, adds nuance to the assessment. Emphasising the importance of a multi-author approach to address potential expert biases and variations in interpretations, the study highlights the effectiveness of using multi-author consultations, incorporating expert judgments, and adopting a dynamic approach for ongoing updates. This approach not only effectively addresses uncertainty but also improves reliability in species evaluations. This comprehensive methodology allows for better-informed choices and actions, fostering effective and transparent dialogues among stakeholders. The practical applicability of the protocol is demonstrated through the Chilean black urchin case study, highlighting its flexibility and suitability for data-limited situations. While recognising uncertainties, the protocol could benefit from explicit quantification, such as assigning confidence levels or ranges to factors. Additionally, the suggestion to enhance the scoring system's qualitative nature by assigning weights to factors based on their impact on uncertainty presents an improvement opportunity. The importance of efficient detection systems and proactive management to ensure accurate declarations and respond to potential threats effectively is also highlighted. Furthermore, incorporating a sensitivity analysis could enhance the identification of factors significantly impacting the assessment. Overall, the protocol is a comprehensive and practical methodology that allows for informed decisions, fostering effective and transparent dialogues among stakeholders, and advancing species conservation and management.

2.7. Acknowledgements

Thank you to the workshop attendees at the National Symposium on Biological Invasions 2017, hosted by the South African National Botanical Institute, for their valuable contributions and active participation in developing this protocol and methodology.

2.8. Appendices

Appendix 2.1: Detailed equation scoring guide.

P (likelihood all populations have been lost)

This factor considers the temporal context of the evidence, and a higher score implies a greater likelihood of population loss, while a lower score suggests a higher probability of population persistence. A high score reflects a substantial time gap since the last evidence of the species, indicating a higher likelihood of population loss, especially in the absence of recent reports or ongoing monitoring. Conversely, a lower score is applied for relatively recent evidence, frequent observations, and active monitoring, suggesting a lower likelihood of population loss.

Higher scoring:

- If there is substantial evidence and high confidence that all populations of the species have been lost. This could include data from comprehensive surveys or studies that suggest the extinction of the species in the region.
- When multiple independent sources or studies confirm the loss of all populations, this adds significant credibility and justifies a higher score. It reflects a strong consensus within the scientific community.
- If a considerable amount of time has passed since the last population was reported or observed, and there is no evidence of persistence, a higher score is suitable. This extended duration increases the confidence in population loss.

Lower scoring:

- If there is limited or inconclusive evidence regarding the loss of all populations, a lower score is justified. It implies that there is uncertainty or a lack of substantial data to support the claim.
- When there are relatively recent reports or observations of the species in the area, even if infrequent, this suggests the possibility of persisting populations and justifies a lower score.
- If there are ongoing monitoring efforts indicating the species might still be present, this implies a lower likelihood of population loss and warrants a lower score. Continuous monitoring efforts suggest that there may be doubts regarding population extinction.

- In cases where there are gaps in data or difficulties in obtaining information, it may not be possible to confidently assert the loss of all populations, leading to a lower score.
- If there is scientific disagreement regarding the extinction of all populations, it reflects uncertainty and justifies a lower score.

F(how long ago evidence of presence was)

This factor considers the time frame between the most recent evidence of presence and the present day. A higher score is allocated when there has been a substantial duration since the last report or evidence of the species' presence, indicating a higher likelihood that the species is no longer present. Conversely, a lower score is justified when the evidence of presence is relatively recent or when there is ongoing or frequent evidence supporting the species' presence.

Higher scoring:

- If the most recent evidence of presence dates back a long time (e.g. several decades or more), it suggests that the species has not been observed or reported for an extended period. This extended duration increases the likelihood that the species is no longer present.
- Significant gaps between reports or observations of the species, indicating sporadic or infrequent occurrences, could suggest that the species is not currently established or widespread in the area.
- A lack of recent sightings or reports, coupled with no ongoing monitoring efforts to document the species' presence, can further strengthen the case for a higher score. The absence of recent evidence suggests a decline or absence of the species in the recent past.

Lower scoring:

- If there is relatively recent evidence of the species' presence (e.g. within the last few years), indicating recent observations or reports.
- If there are ongoing or frequent observations or reports of the species, even if they are intermittent, suggesting the species is present or has the potential to persist.
- If continuous monitoring efforts are in place, with recent reports or observations documented through systematic surveys or citizen science initiatives, providing confidence in the species' continued presence.

Overall, the scoring for *F(how long ago evidence of presence was)* should reflect the temporal context of the evidence and how recent or frequent the species' presence has been observed or reported. The higher the score, the greater the likelihood that the species is no longer present in the area, while a lower score indicates a higher probability of current or recent presence.

F(search effort)

The scoring for *F(search effort)* is based on the thoroughness and effectiveness of the search efforts made to determine the presence or absence of the reported species. A higher or lower scoring for search effort depends on the quality and extent of the search activities conducted. Here are some factors that can justify a higher or lower scoring for *F(search effort)*:

Higher scoring:

- Extensive and comprehensive surveys: If the search efforts involve extensive surveys that cover a wide range of potential habitats and locations where the species could potentially be found, it justifies a higher scoring. Comprehensive surveys increase the likelihood of detecting the species if it is present.
- Involvement of experts and specialists: When experienced individuals, experts, or specialists in the field are involved in the search efforts, it adds credibility and increases the accuracy of the survey. Their knowledge and expertise can ensure that the survey is conducted effectively and that any potential signs of the species' presence are properly recognised and recorded.
- Use of modern techniques and technology: Employing modern surveying techniques, such as DNA analysis, remote sensing, camera traps, and other advanced technologies, can significantly improve the chances of detecting the species, especially if it is elusive or difficult to observe.
- Multiple and repeat surveys: Conducting multiple surveys over a considerable period allows for more opportunities to detect the species, particularly if it has sporadic or seasonal occurrences. Repeating surveys also helps validate the results and reduces the likelihood of missing the species due to chance.

Lower scoring:

- Limited geographic coverage: If the search efforts are confined to a small or limited geographic area, it reduces the chances of detecting the species.
- Insufficient survey duration: If the search efforts are conducted for a short period, it may not be enough to account for variations in the species' population or occurrence over time. Some species might have sporadic occurrences, and a short survey duration may miss their presence during certain periods.

- Lack of expertise: If the survey team lacks expertise or experience in identifying the reported species or the ecological conditions under which it thrives, it may lead to misidentification or overlooking potential signs of its presence.
- Inadequate sampling effort: If the number of samples collected or observations made during the search efforts is insufficient, it reduces the statistical reliability of the results and may miss the presence of the species.

In summary, a higher scoring for *F(search effort)* is justified by extensive and comprehensive surveys, involvement of experts, use of modern techniques, and multiple repeat surveys. Conversely, a lower scoring is justified by limited geographic coverage, insufficient survey duration, lack of expertise, and inadequate sampling efforts. The higher the scoring for *F(search effort)*, the more confidence we can have in the conclusion regarding the presence or absence of the reported species.

F(certainty of identification)

The scoring for *F(certainty of identification)* depends on the level of confidence and reliability in the identification of the reported species. A higher scoring for certainty of identification (e.g. high or medium) would be justified when there is substantial and reliable evidence supporting the species' identification, reducing the likelihood of misidentification or false positives.

Higher scoring:

- Multiple independent confirmations: If the reported species has been independently identified and confirmed by multiple experts or researchers, it increases the confidence in its accuracy.
- Detailed and comprehensive evidence: The availability of detailed information, such as clear images, physical specimens, or DNA analysis, can enhance the certainty of the species' identification.
- Expertise and familiarity: If the person or team making the initial report is highly knowledgeable and familiar with the species, it adds credibility to the identification.
- Recognition from reputable sources: If the report has been recognised and endorsed by reputable organisations or institutions specialising in biodiversity, it strengthens the case for accurate identification.

On the other hand, a lower scoring for *F(certainty of identification)* (e.g. low) would be justified when there are significant uncertainties or shortcomings in the identification process, raising concerns about the possibility of misidentification.

Lower scoring:

- Limited supporting evidence: If the reported species is solely based on human observation without additional corroborating evidence (e.g. images, physical specimens, or DNA analysis), it may raise doubts about its accuracy.
- Lack of expertise: If the person making the initial report is not an expert in the field or lacks familiarity with the species, it may increase the likelihood of misidentification.
- Similar-looking species: The presence of similar-looking species in the region may lead to confusion and misidentification of the reported species.
- Lack of verification: If the initial report has not undergone peer review or validation by other experts, it may raise questions about its reliability.

To summarise, a higher scoring for *F(certainty of identification)* when calculating *P(absence)* is justified when there is strong evidence and confidence in the accuracy of the species' identification. Conversely, a lower score is appropriate when there are uncertainties, limitations, or doubts about the identification process, which may affect the reliability of the absence assessment. The level of certainty in the identification plays a pivotal role in determining the overall probability of absence.

F(quality of samples or evidence)

The scoring for *F(quality of samples or evidence)* would be justified based on the reliability, comprehensiveness, and accuracy of the available evidence or samples. A higher scoring for *F(quality of samples or evidence)* would be appropriate if the evidence and samples supporting the absence claim are strong, well-documented, and come from reputable sources.

Higher scoring:

- Comprehensive surveys: If the absence claim is supported by comprehensive surveys conducted by experts over a wide area and for a significant duration, it adds credibility to the assessment.
- Reproducibility: High-quality evidence would be more reliable if the same absence conclusion can be replicated independently by other researchers or experts using similar methodologies.
- Reputable sources: If the evidence is from reputable scientific journals, governmental reports, or institutions with a track record of reliable research, it enhances the quality of the evidence.
- Multiple data points: Having multiple data points or independent reports supporting the absence claim strengthens the argument.

- Data transparency: High-quality evidence should include detailed information about the methodologies used, GPS locations, images, and other relevant data, allowing for scrutiny and verification.
- Expert input: If the evidence is reviewed and supported by subject-matter experts in the field, it adds credibility to the assessment.

On the other hand, a lower scoring for *F(quality of samples or evidence)* would be justified if the available evidence is limited, lacks detail, or suffers from methodological flaws that could introduce uncertainty or bias into the assessment.

Lower scoring:

- Limited data: If the evidence is based on a single or limited number of reports or observations, it may not be sufficient to draw strong conclusions about the absence of the species.
- Incomplete records: If the available records are lacking in critical information, such as GPS locations, images, or detailed descriptions, it may reduce the reliability of the evidence.
- Ambiguity: If the evidence is ambiguous or open to different interpretations, it may lead to doubts about the accuracy of the absence claim.
- Lack of expert review: If the evidence has not been reviewed or verified by subject-matter experts, it may raise concerns about its reliability.
- Conflicting evidence: If there are conflicting reports or evidence that suggest the species may still be present, it could weaken the absence claim.

In conclusion, a higher scoring for *F(quality of samples or evidence)* when calculating *P(absence)* is justified by strong, comprehensive, and reliable evidence from reputable sources, while a lower scoring is justified by limited, incomplete, or uncertain evidence that may introduce doubts about the absence claim. It is essential to consider the quality of the evidence carefully to make well-informed decisions.

3. Where are they now? *Calluna vulgaris* and *Euphorbia esula* in South Africa as case studies.

3.1. Abstract

A country's list of alien species should be based on recent verified field observations and curated physical specimens with accompanying morphological and molecular identification. In practice this is not always possible, resulting in errors in such lists. These inaccuracies can lead to spurious species counts, confusion, and inefficient allocation of resources. This study addresses one aspect of this issue—that of declaring an alien species as absent from a country despite previous evidence of presence. The protocol developed in Chapter two was applied to *Calluna vulgaris* and *Euphorbia esula* (listed alien plant species with disputed presence) in South Africa. *Calluna vulgaris* was assigned a medium probability of absence, necessitating further investigation, while *E. esula* received a high probability of absence. After field surveys, *C. vulgaris* was found, while *E. esula* was not, confirming the protocol's effectiveness by supporting the probabilities of absence but urging proactive measures. Despite limitations, the protocol showed a high degree of accuracy and predictive capability. The risk analyses resulted in high potential impacts for both species, and recommended a listing of Category 1a for *C. vulgaris* and consideration of a prohibited listing for *E. esula*. The outcomes underscore the complexities of species assessment, emphasising nuanced management strategies. This study unveils the protocol's utility in simplifying the challenges of accurately listing alien species as present or absent in a country. It also emphasises the significance of robust data collection for effective management of alien species.

3.2. Introduction

Inaccurate assessments of species presence and premature removal from lists can have significant consequences for national eradication efforts (Henderson & Wilson, 2017). These decisions, influenced by various factors, can lead to repercussions, such as inaccurate species accounts and thus the misallocation of valuable management efforts and resources (Wilson et al., 2017). For more reliable and precise lists of alien species, it is preferable that they are based on recent field observations, accompanied by the physical curation of specimens. These specimens should undergo rigorous identification processes, including morphological and molecular analyses (Magona et al., 2018). Unfortunately, in practice, such comprehensive approaches are often overlooked, leading to inaccuracies in these lists. Errors in these lists may arise from mistaken declarations of species as absent. This can occur due to misidentifications, such as when species are only identified at the genus level, confused with native species, or bear resemblance to other alien species that are already recorded as present (Carlton, 1996; Ceschin et al., 2016; Clusa et al., 2018; Marble & Brown, 2021; Pyšek, 2003; Pyšek et al., 2012; Saltonstall, 2002; van Wilgen et al., 2020). According to MacKensie et al. (2017), alien plant species with subtle traits and smaller populations are prone to misidentification, while those with conspicuous features and larger populations are more likely to be correctly identified. Similarly, species resembling native plants or having limited spread may remain undetected, leading to delayed intervention (Verloove, 2010; Marble & Brown, 2021). Undetected species can lead to further invasions, emphasising the importance of accurate identification and distribution data reporting for all alien species (Panetta & Lawes, 2005).

Many unintentionally introduced species are likely under-recorded in current listings due to understudied groups or a lack of taxonomic knowledge (Cohen et al., 2002; Pyšek et al., 2012). This oversight extends to well-known taxa like the Australian genera Melaleuca (e.g. Jacobs et al., 2017; Matthys et al., 2022) and Acacia in South Africa (e.g. Magona et al., 2018). The introduction of certain species may not be immediately detected as they take time to establish and become invasive, with a span between introduction and naturalisation that can extend over decades (Crooks, 2005; Richardson & Pyšek, 2012). Lag durations for invasiveness can vary due to ecosystem or environmental changes (Marble & Brown, 2021), causing a delay in population growth and often leading to species going unnoticed during surveys (Crooks et al., 1999; Essl et al., 2018). Some alien species may also exhibit 'boom-and-bust cycles,' marked by rapid growth followed by decline, and spontaneous collapse can occur even without management efforts once a species is well-established in its invasive phase (Cooling et al., 2012; Simberloff & Gibbons, 2004; Strayer et al., 2017). Species can also be introduced but fail to become invasive due to factors including low propagule pressure, abiotic and biotic resistance, genetic constraints, and mutualist release (Senni and Nuñes, 2013; Qongqo et al., 2022).

Another possible reason for being considered absent is an underestimation of species that are present in the country during initial surveys. For example, Visser et al. (2017) updated the alien grass species inventory for South Africa using recorded occurrences from literature databases. It was determined that 256 alien grass species are present in the country; however, a preliminary list of alien grass species produced by Milton (2004) stated that there were 113 alien grass species present in South Africa. Given many of the taxa recorded by Visser et al. (2017) were introduced prior to 2004, this example shows how sensitive the timeframe of such lists is to detection and recording efforts. Another example is in Hawaii where a survey of alien arthropods discovered 490 alien species, including 145 new records for Maui (Loope & Howarth, 2002). However, 40% of these had been in Hawaii for at least 50 years and were likely on Maui for extended periods of time before being discovered (Loope &

Howarth, 2002). Some alien species go unnoticed and are mistakenly assumed absent due to inadequate monitoring (Cohen et al., 2002) and be falsely considered absent (Bailey et al., 2004; de Solla et al., 2005; Kery, 2002, Kéry & Gregg, 2003; Slade et al., 2003; Tyre et al., 2003; Wintle et al., 2004). Some species can exhibit cryptic traits, disappearing and reappearing at specific locations within short periods (Garrard et al., 2008). Therefore, in order to prevent such alien species from becoming invasive, it is best to implement regular monitoring for invasive species in order to detect them and intervene as soon as possible (Maxwell et al., 2009). Multiple methods are used to monitor and detect alien species but state agencies, citizen scientists, land owners and other groups often monitor invasive plant spread using conventional monitoring methods such as field observations (Foxcroft et al., 2008). One strategy for determining the presence of a species is to continuously visit the location where the species was reported until one or more individuals are discovered, or to discontinue the search after a series of unsuccessful visits and consider the species as no longer present at that location (Kery, 2002). Traditional monitoring methods are for the most part precise and effective, but impractical at large spatial scales because it can become time and cost intensive. Difficulties of access to isolated places can also hinder traditional monitoring approaches (Royimani et al., 2019). Unmanned aerial vehicle footage is becoming more common in monitoring because it overcomes labour and access difficulties (Papp et al., 2021) but expense, technical knowledge, and species with a distinctive spectral signature or dominating canopy structure may restrict monitoring efforts (Rodgers et al., 2018). In large biodiverse countries with limited resources, like South Africa, this becomes even more of a challenge (but see Duncan et al., 2023 and Newete et al., 2023.).

In South Africa, there are nationally listed plant and animal species, like *Calluna vulgaris, Euphorbia esula* (Henderson and Wilson, 2017) and *Tetrapygus niger* (Mabin et al., 2015) that have disputed presence data and may possibly not be in the country at all. For species like this, a prohibited species list was created, based in part on expert opinion, as part of national regulations on alien and invasive species (Department of Environmental Affairs, 2016). Species on the prohibited list were believed to not be present in the country and whose introduction should be prevented (Department of Environmental Affairs, 2016). Both *Calluna vulgaris* and *Euphorbia esula* were listed as alien species on the most recent Alien & Invasive Species List (Department of Environmental Affairs, 2020) and based on their regulatory listing recorded as present on the Global Register of Introduced and Invasive Species for South Africa (GRIIS) for South Africa (Robinson et al., 2020 & 2020a). Both species also have disputed presence data as they have not been recorded with physical evidence and confirmed as present since the first anecdotal reporting, and therefore may not be present in the country (Henderson and Wilson, 2017). Currently, South Africa does not have a protocol to classify such alien species as absent despite previous evidence of presence, which presents challenges when it comes to revising or reclassifying these species in national legislation. This chapter applies the protocol developed in Chapter two to two alien plant species, *C. vulgaris* and *E. esula*, with disputed presence in South Africa. Therefore, the aim of this chapter is to test the implementation of the protocol through: 1) examining historical documentation of *C. vulgaris* and *E. esula* in South Africa; 2) applying and testing the protocol developed in Chapter two; 3) assessing the accuracy of the protocol's results; and 4) evaluating potential risks and providing management recommendations.

3.3. Research Design and Methodology

3.3.1. Study area and study species

This study includes South Africa as its primary study area, with a particular focus on regions where the case study species have been reported.

Calluna vulgaris (heather) is an evergreen shrub common in Northwest European heathlands (Tutin et al., 1972). Native to regions with cold winters, it thrives in frost-covered lowlands, thickets, and higher clustering grasslands, at elevations of up to 1600 meters (CRC Australian Weed Management, 2003; Chapman & Bannister, 1990). Heather produces flowers in various shades of pale purple, pink, or white on short stalks (Gimingham, 1960). It forms long-lasting soil seed banks, with 20 to 32 seeds per capsule (Gilbert & Butt, 2010) and tens of thousands of viable seeds per square meter (CRC Australian Weed Management, 2003). Germination takes 8–14 days under ideal conditions (Gimingham, 1960), and seedlings often grow in nutrient-poor soil (Gimingham, 1994). It has been intentionally planted in several countries for various purposes (Monschein et al., 2010; Global Invasive Species Database, 2020) and has been reported in regions like the Falkland Islands, the Crozet Islands (Chapman & Bannister, 1990; CRC Australian Weed Management, 2003), and South Africa's fynbos biome (iNaturalist.org., 2022). In South Africa, it was previously listed as a prohibited species (Department of Environmental Affairs, 2016).

Euphorbia esula, commonly known as leafy spurge, is a European and Asian native (Morrow, 1979; Rhoads & Block, 2011). It's a perennial plant, up to 80 cm tall, with woody crown roots and non-woody, hairless stems (Global Invasive Species Database, 2021). Its lanceolate leaves are frosted in bluish green to green hues, arranged alternately (Global Invasive Species Database, 2021). Flowers cluster at the stem's top, most noticeable during peak flowering when bracts change colour (Global Invasive Species Database, 2021; Messersmith et al., 1985). Leafy spurge thrives in diverse habitats, from waterways to uplands, tolerating temperatures of 10°C to 37°C and annual precipitation from 178 mm to 635 mm (St. John & Tilley, 2014). Explosive seed capsules disperse seeds around the parent plant (Hanson & Rudd, 1933; Bakke, 1936; Selleck et al., 1962). One stem yields about 252 seeds viable for 5 to 8 years, with 60% to 80% germination success (Selleck et al., 1962; Global Invasive Species Database, 2021). It possesses invasive potential, displacing native plants, and is an eradication target in South African legislation as it was reported to be present in the country (Bangsund et al., 1999; St. John & Tilley, 2014; Wilson, 2023). However, there are some disagreements or uncertainties regarding the accuracy of the data indicating its presence (Henderson & Wilson, 2017).Record surveys and literature reviews of the species' historic accounts

A comprehensive historical account of the presence and occurrences of both study species in South Africa was compiled. These historical data serves as background and a reference for the argument map. A literature review was also completed for the species to determine the probability that it is absent from the country despite some previous evidence of presence. Detailed information of each case study species was collected to assess the probability of introduction, presence, and/or disappearance of the species in the country. To achieve this, information from herbarium records, museums, botanical gardens, and grey literature was gathered and put into a timeline to create a historic account. The information that was gathered includes viable and operating pathways of introduction (past, present, and future) as well as the last time the species was recorded and confirmed as present and how it was managed (if at all).

Location data were collected and records of presences of both study species in South Africa were collated. This was done by running a search on the Global Biodiversity Information Facility (GBIF) (www.data.gbif.org), an on-line spotter network (www.iNaturalist.org), GenBank (https://www.ncbi.nlm.nih.gov/genbank/), the database of herbarium records (www.newposa.sanbi.org), the Web of Science, and on Google Scholar using the species name and "South Africa" for all listed species to see which have physical evidence or historical records of being present in the country. The following scientific names were taken into account during the examination of records for *Calluna vulgaris* (L.) Hull: *C. atlantica*, *C. belesiana*, *C. ciliaris*, *C. elegantissima*, *C. erica*, *C. genuina*, *C. sagittifolia*, *Erica ciliaris*, *E. confusa* Gand., *E. glabra*, *E. herbacea*, *E. lutescens*, *E. nana*, *E. prostrata*, *E. reginae*, *E. sagittifolia*, *E. vulgaris*, and *E. vulgaris* (Wfoplantlist.org, 2021). For leafy spurge, the scientific names considered were *Euphorbia esula*, *E. gmelinii* (Steudel), *E. intercedens*, *E. podperae*, *E. virgata*, *E. shiguliensis* (Schur), *Galarhoeus esula* (Wfoplantlist.org, 2021), and *Tithymalus esula*, as documented by Dunn (1979).

Local experts and conservation officers were consulted, and the potential localities they suggested were visited to confirm the presence or absence of these species. Search/ survey history and effort was determined by requesting reports, records and GPS data for any efforts conducted by the South African National Biodiversity Institute (SANBI).

3.3.2. Calculating the probability that an alien plant species is absent from country

An argument map was compiled for each species using the information obtained in the literature reviews.

3.3.3. Field surveys

The perimeter of the *C. vulgaris* population was established by systematically walking and surveying the area ~100 m from the last plant encountered to ensure that the extent of the population was accurate. The location for *E. esula* was visited and systematically surveyed using parallel walked transects with three surveyors spaced ~5 m apart to detect and map all plants. Since the reported location of *E. esula* was on the bank of a river, these systematic walks were done from the reported location in each direction of the river. All nearby properties and public open spaces along the river were also surveyed. Other than these systematic searches, a random search in potentially suitable habitats (i.e. habitats which are known to be or may be potentially favourable for *E. esula* and *C. vulgaris*) was done for more populations, outlier plants, or similar-looking species.

3.3.4. Risk analysis and regulatory recommendations

Risk analyses were conducted for both study species using the framework of Kumschick et al. (2020b). The risk analysis framework used is specifically designed for the purpose of listing alien species under the regulatory protocol of the South African National Environmental Management: Biodiversity Act 10 of 2004 Alien and Invasive Species Regulations (NEM:BA). The findings of this evaluation were used to make management recommendations for the case study species. These recommendations were used to address the implications of the protocol's findings and include preventive, management, and/or eradication efforts, depending on the level of risk associated with each species.

3.4. Results

3.4.1. Testing the protocol on *Calluna vulgaris*

i. Step 1: Literature review and historic account

The first evidence of *C. vulgaris* in South Africa is a preserved specimen in the National Museum of Natural History, Smithsonian Institution and was recorded in 1987 in the Western Cape, South Africa (Orrell, 2023) (Figure 3.1). The record reflected that it was published without coordinates, but included a textual description of its location. The description of the location however was not detailed as it stated the following: “Caledon District, Riviersonderend, flats on bank of river near the town”. It is recorded under the applied name at the time, *Erica confusa* Gand. and does not contain any associated media of the specimen, such as photographs, illustrations, or other visual representations. There was no evidence reported or record for *C. vulgaris* for the next 19 years and the next report of the species was in 2011 even though it was observed in 2008 (iNaturalist, 2023a) (Figure 3.1). It was documented within the Table Mountain National Park, situated in the Peninsula sandstone fynbos habitat, identified by coordinates -33.971578,18.421646 (iNaturalist, 2022b; Rebelo et al., 2006) (Figure 3.1). The third report was in 2021 (Figure 3.2), in the same location as the previous record (iNaturalist, 2023a). *Calluna vulgaris* was searched for in 2011, the same year as the first citizen scientist report (Figure 3.2), as well as in 2012 by SANBI but was not found. It was searched for once again by the author as part of this study following the report in 2022 and was indeed found (Figure 3.1). There were no fires on record since 1962 (earliest records available) for the -33.971578,18.421646 locality *C. vulgaris* was reported at (SANParks, 2022).

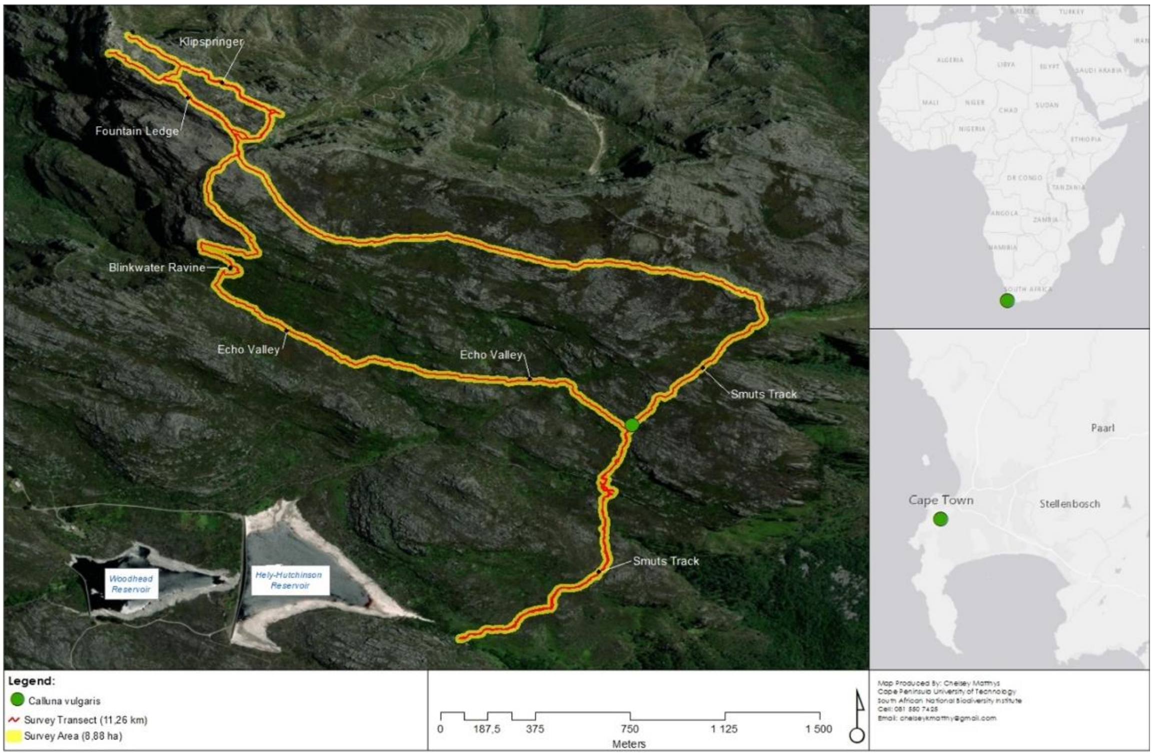


Figure 3.1: Map indicating the reported location and search efforts of *Calluna vulgaris* in Western Cape, South Africa by Matthys, 2022.

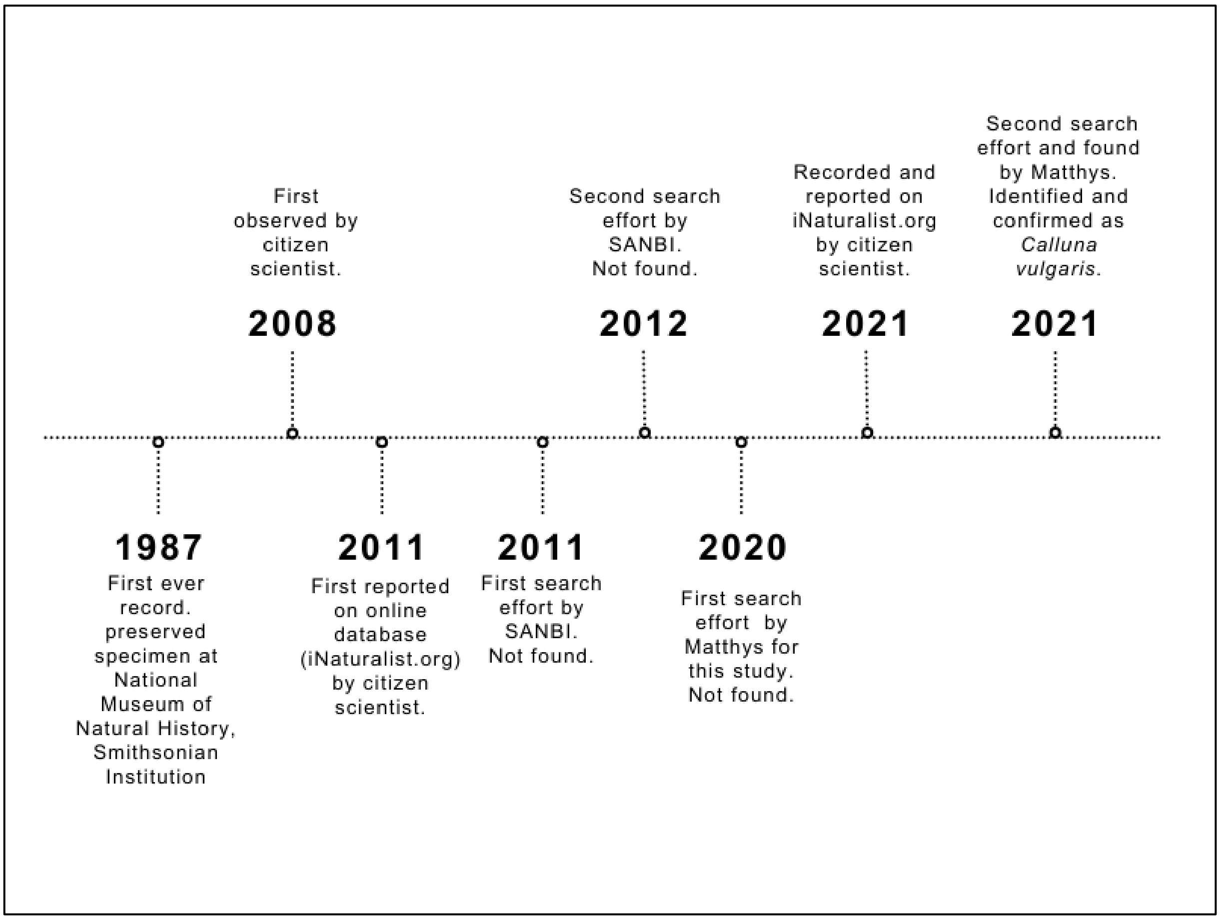


Figure 3.2: A narrative of the history of Calluna vulgaris in South Africa.

ii. Step 2 & 3: Calculating the probability of absence

The probability of the absence of *C. vulgaris* in the country was assessed through a comprehensive argumentative approach, drawing upon insights from the literature review and existing knowledge. This evaluation is visually represented in the argument map presented in Figure 3.3. Within the argument map (Figure 3.3), the proposed 'absent claim' of the species from the country is established, suggesting that *C. vulgaris* is absent from the country. The argumentative approach considered various factors, including the species' historical records, potential misidentifications, and the reliability of available evidence. This analytical process aimed to provide a well-founded and substantiated perspective on the presence or absence of *C. vulgaris* in the given geographic context. Using the information provided in the argument map and literature review, the equation underwent scoring. This analysis evaluated how specific factors and rationale presented in the argumentative approach contributed to the weighted evaluation and eventual determination of the probability of *C. vulgaris'* absence.

P(it has not disappeared)

P(likelihood all populations have been lost)

'Reason 1' asserts that search efforts have been adequate, backed by reports from formal search endeavours and multiple attempts by experienced individuals. Nevertheless, 'Objection 1' introduces a significant counterpoint, citing the poor records of search efforts and the absence of GPS location data. This lack of precise location information raises questions about the thoroughness of the searches, thereby creating uncertainty. The objection holds more weight, leading to a low probability score, indicating some ambiguity regarding the timing of evidence. 'Reason 2' points to the shortcomings of historical reports, citing the extended gaps between observations (1987, 2008, 2011, 2021; Figure 3.3). However, 'Objection 2' strengthens the argument for the quality of historical evidence as the most recent was in 2021 by a citizen scientist, months prior to the evaluation of the species using this protocol. In this context, the objection carries more weight, asserting confidence in the reliability of historical records, resulting in a low probability of presence score. 'Reason 4' suggests that the absence of records of disturbance (specifically by fire) at a reported location indicates a low likelihood of new or re-introductions as the species is fire-driven. Contrarily, 'Objection 4' argues that the site remains favourable for new and re-introductions due to the plant's high seed production, viability, and the accessibility of reported locations. Essentially, the plant's ability to produce a large number of viable seeds, coupled with the ease of access to reported locations by humans, makes it more likely for the plant to establish or reintroduce itself in that particular area. The objection, supported by these factors, challenges the notion of total population loss. Consequently, the objections carry more weight, yielding a low probability score, signifying a notable likelihood of populations persisting.

P(it was present)

F(certainty of identification) and F(quality of evidence/sample)

'Reason 2' highlights the inadequacy of the historical records, primarily due to long intervals between observations, imprecise GPS location data, and the absence of media images for preserved specimens. This diminishes the overall reliability and comprehensiveness of the historical records, particularly concerning media images, which serve as visual evidence supporting or supplementing textual information and thereby enhancing the records' reliability and comprehensiveness. However, 'Objection 2' reinforces the argument by presenting a recent, well-documented 2021 report with GPS data and images. This recent evidence significantly enhances the quality of samples and supports *Calluna vulgaris*' identification. The probability score will be high, reflecting high confidence in recent evidence, despite earlier deficiencies. 'Reason 3' raises concerns about misidentification, citing a preserved specimen lacking GPS coordinates and the presence of similar-looking species. In contrast, ‘Objection 3’ strengthens the case for identification certainty, pointing to a preserved specimen recorded by the Informatics Office at the National Museum of Natural History and a detailed 2021 record with GPS data and images from two different observers. As a result of the supporting evidence, the ‘Objection 3’ carries more weight. Hence, the probability score is high.

P(absence)

Based on the results of the evaluation above, the overall probability of absence leans toward a medium probability of absence

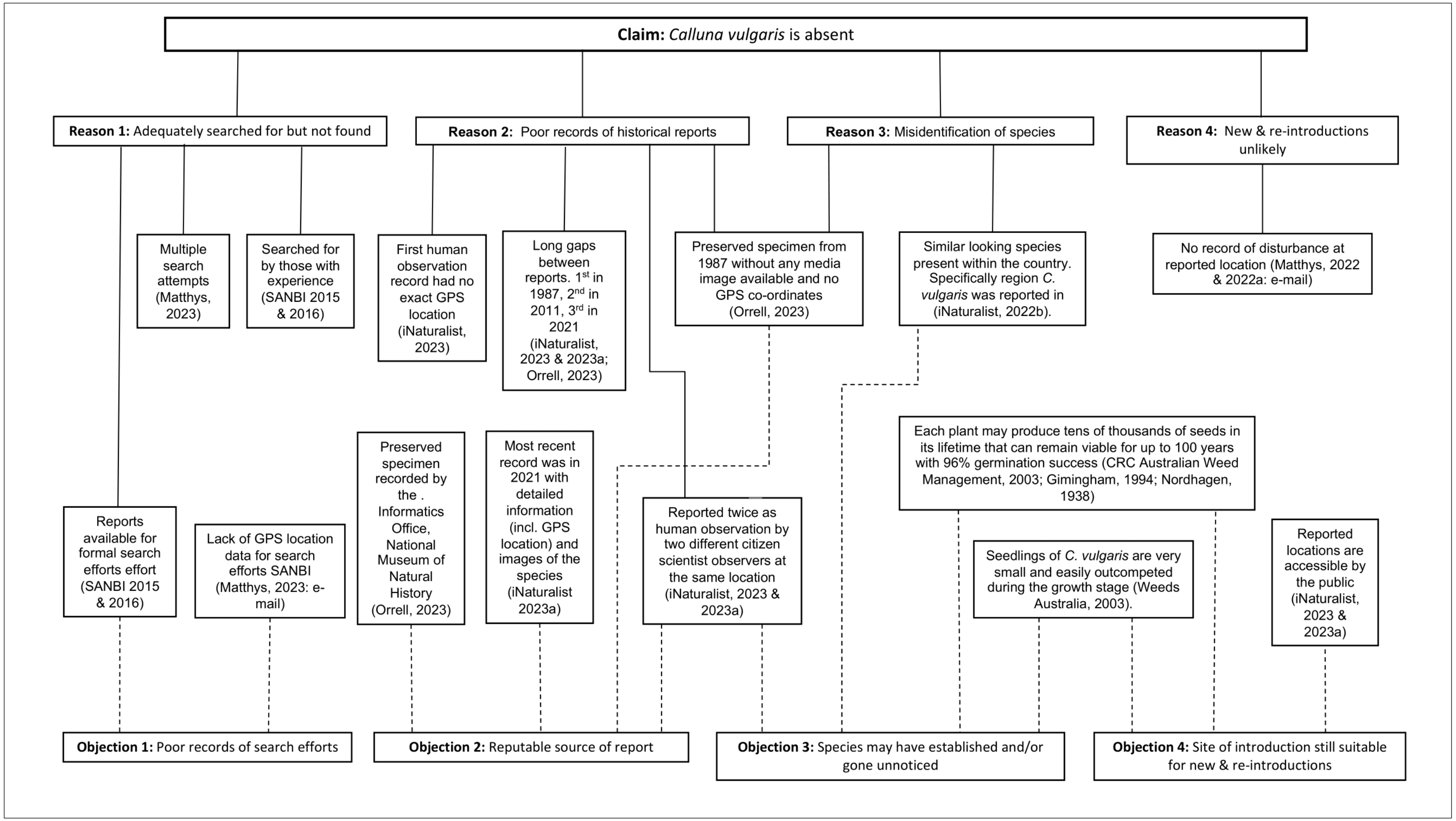


Figure 3.3: Argument map for the claim that *Calluna vulgaris* is absent from South Africa. The claim appears in the uppermost box titled 'Claim' and is based on various reasons, shown in boxes titled 'Reason' and each reason is supported by evidence. 'Objection' to the evidence and by inference, the reasons, are shown in boxes titled 'Objection'.

iii. Field survey for Calluna vulgaris

In 2021, *C. vulgaris* underwent the proposed protocol, initiating two search attempts to assess the protocol's accuracy. The initial search yielded no results, prompting considerations that the species might have been misidentified as a similar-looking native plant, *Erica hirtiflora* was found in close proximity to the reported location (Figure 3.4A). However, during the second search attempt, a detailed report on iNaturalist.org.za by a citizen scientist provided better information, including coordinates and images, leading to the successful discovery of a single individual *of C. vulgaris* (Figure 3.4B) within mature fynbos vegetation (Figure 3.4C). This finding supports the protocol's usefulness, suggesting its utility in pre-search assessments to determine probability of species absence. Notably, when the protocol indicated a medium probability of absence, subsequent searches revealed the presence of the species, affirming the need for thorough reporting of species characteristics and observations for accurate identification.

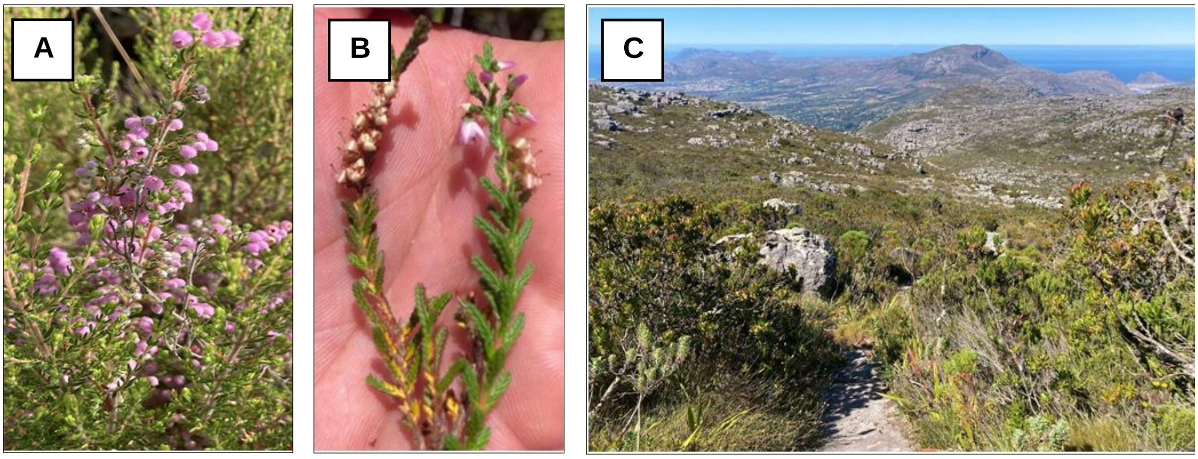


Figure 3.4: Evidence from field surveys for *Calluna vulgaris*. **A.** *Erica hirtiflora* in flower found on Table Mountain (native); **B.** Flowering *C. vulgaris* found on Table Mountain, South Africa; **C.** The location in which **D.** vulgaris was reported by a citizen scientist in 2021 and subsequently found for this study.

3.4.2. Risk analysis for *Calluna vulgaris*

Despite having only one confirmed record in South Africa, *C. vulgaris* received a high-risk score due to its invasive potential, leading to habitat domination and displacement of native plants (Appendix 3.1). This risk score is a result of the combination of a major score for consequences of risk and an unlikely score for likelihood of risk (Kumschick et al., 2020a). Even though *C. vulgaris* seedlings are small and easily outcompeted, as they mature, adult plants form a dense canopy and persistent leaf litter (CRC Australian Weed Management, 2003). While the likelihood of new introductions is low to stringent biosecurity measures, the species has suitable climate and habitats in South Africa, and there is a risk of dominance, indicating its potential to outcompete native species and become the predominant vegetation in fire-driven ecosystems. In light of these findings, it is recommended that *C. vulgaris* be listed as a Category 1a species.

3.4.3. Testing the protocol on *Euphorbia esula*

i. Step 1: Background literature review and historic account

The first and only report of *E. esula* was recorded in October 2006 in the Southern African Plant Invaders Atlas (SAPIA) (record number 58885) by an expert, foreign to South Africa (Henderson and Wilson, 2017). It was reported to be present in Gauteng, specifically near the Hennops River, within a green open space adjacent to the river (-25.831000, 28.146646) (Figure 3.5). The first search effort for *E. esula* was in 2011 by the initial observer who reported the species. He had mentioned that the area looked completely transformed from when he reported the species in 2006. Despite multiple searches from 2012 – 2015 and awareness raising amongst surrounding landowners, *E. esula* was not located (Henderson & Wilson, 2017; SANBI, 2015 & 2016) (Figure 3.6).

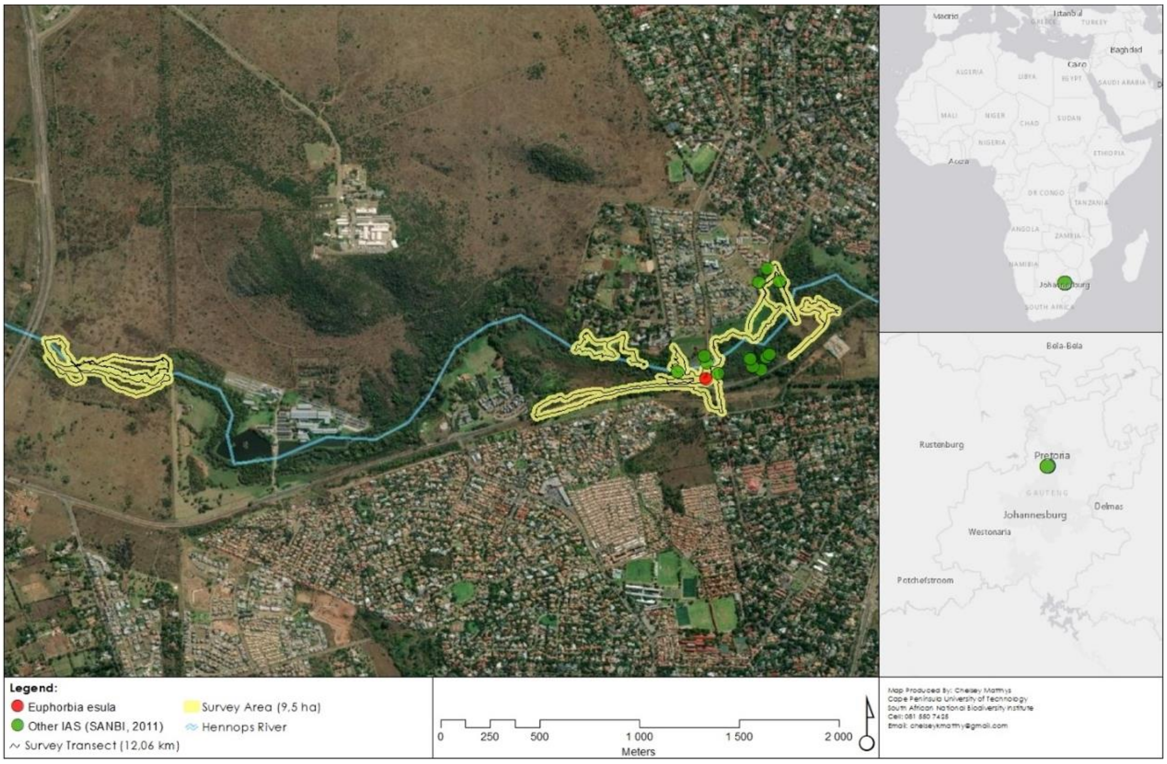


Figure 3.5: Map indicating the reported locations and search efforts of *Euphorbia esula* in Gauteng, South Africa by Matthys, 2022.

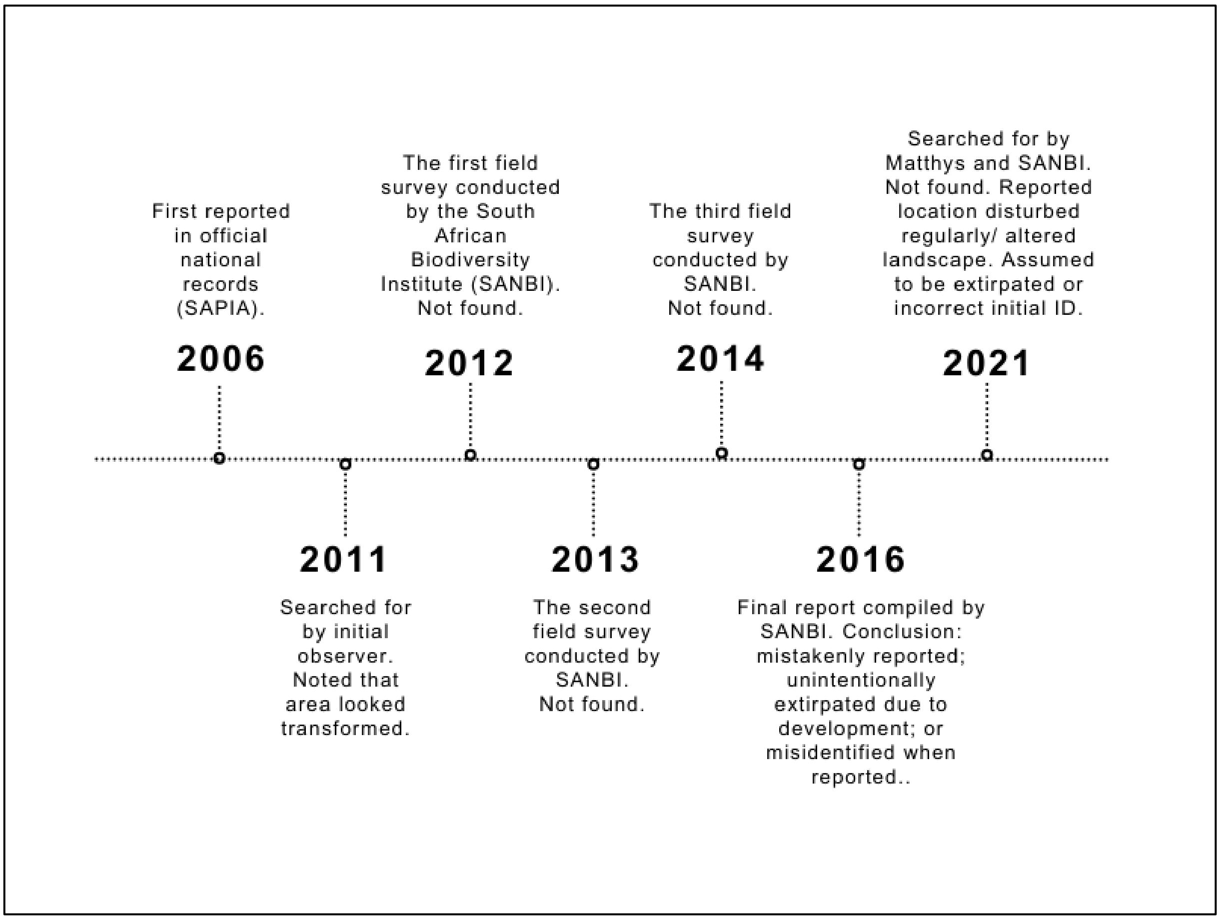


Figure 3.6: A narrative of the history of *Euphorbia esula* in South Africa.

ii. Step 2 & 3: Calculating the probability of absence

The absence probability assessment *for E. esula* in the country employs an argumentative approach, synthesising insights from a literature review and existing knowledge. This evaluation is visually depicted in the argument map presented in Figure 3.7, where the proposed 'claim' posits that *E. esula* is absent from the country. Key considerations within the argumentative approach encompass the examination of historical records, potential misidentifications, and the reliability of available evidence. This analytical process aims to establish a well-founded and substantiated perspective on the presence or absence of *E. esula* in the specific geographic context.

Utilising the information outlined in the argument map, the equation undergoes scoring to assess how specific factors and rationale presented in the argumentative approach contribute to the weighted evaluation. This analysis ultimately determines the probability of *E. esula'*s absence.

P (it has not disappeared)

P(likelihood all populations have been lost)

'Reason 1' concerns the search effort to locate the reported species, with evidence of a 16-year extensive search by experienced individuals and formal initiatives but no sightings were made. 'Objection 1' raises valid concerns about search limitations, including site-specific efforts, absence during peak flowering, limited geographic coverage, and the presence of similar-looking species, casting doubt on 'Reason 1'. 'Reason 1' however carries more weight, warranting a high score for search effort. 'Reason 2' addresses the timing of the last reported sighting in 2006, signifying potential absence. There is no objection to this point, resulting in a high score for how long ago evidence of presence was. 'Reason 4' raises concerns about the potential loss of all populations, supported by restrictions on species introductions, disturbed reported locations, and limited records of introductions. 'Objection 4' counters this with suggestions of ongoing introductions due to active pathways, substantial seed production, and a persistent soil seed bank. Both 'Reason 4' and 'Objection 4' provide valid arguments, meriting a medium score for likelihood all populations have been lost. 'Objection 4' mentions active pathways, potential agricultural introductions, and *E. esula*'s adaptability. The area is transformed, so even if it was there, the likelihood that all populations are lost is high.

P(it was present)

F(certainty of identification) and F(quality of evidence/sample)

‘Reason 3’ questions the certainty of identification, as the initial report relied on human observations without additional proof, further complicated by it being made by a citizen scientist. Conversely, ‘Objection 3’ argues that the citizen scientist was knowledgeable about the species, and the report is documented in a reputable source, strengthening the certainty of identification, but to a limited extent. Both 'Reason 3' and 'Objection 3' hold relevance, warranting a medium score for certainty of identification. ‘Reason 3’ also raises concerns about the quality of the evidence/sample, as the species was reported only once in South Africa without verification, such as a photo or physical specimen. ‘Objection 3’, however, notes its documentation in SAPIA. Nevertheless, due to the limited evidence and absence of verification, a low score is assigned for quality of evidence/sample. This means there is a high likelihood that the species is absent.

P(absence)

Considering the above, the assessment indicates an overall high likelihood of absence.

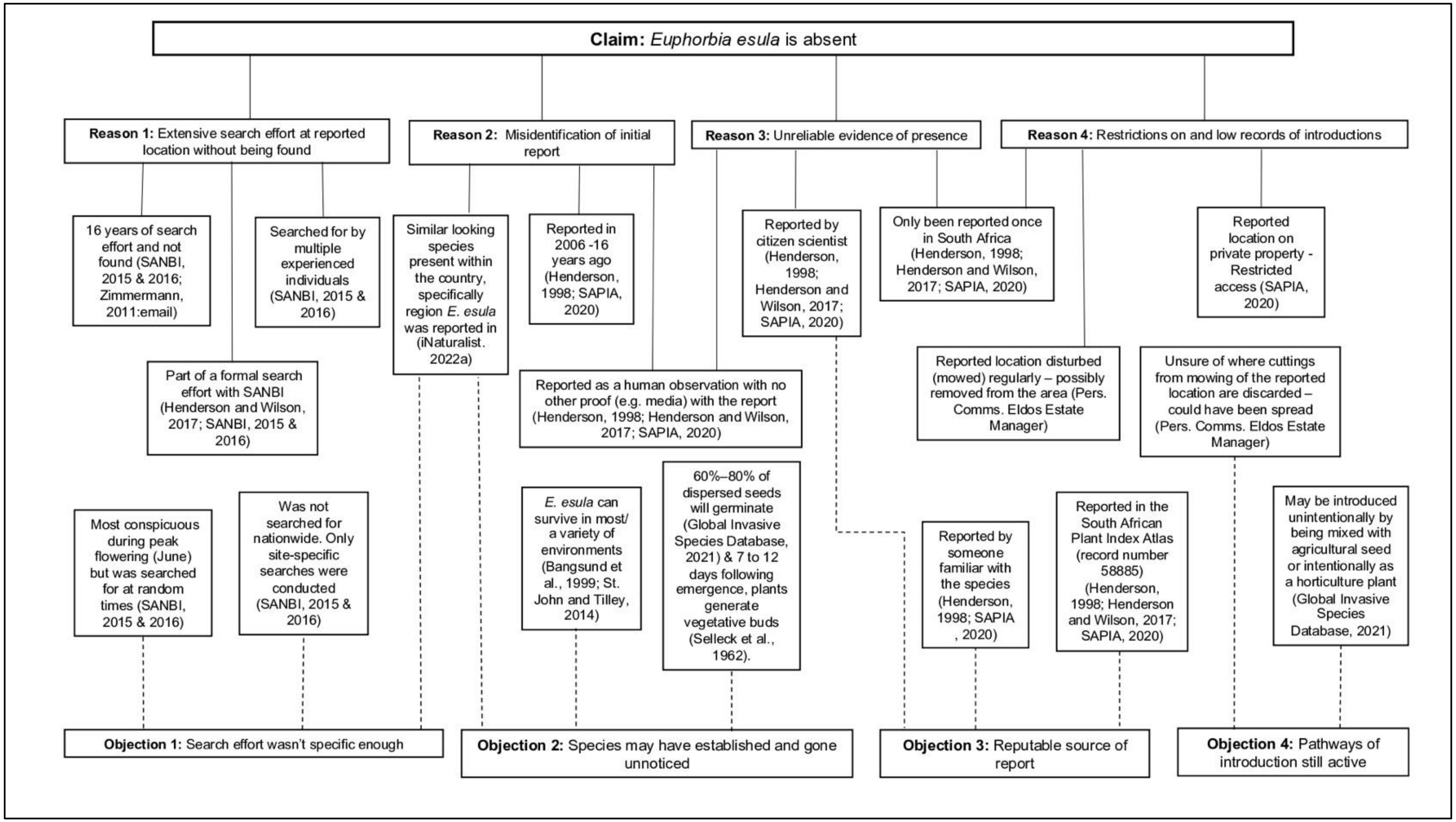


Figure 3.7: Argument map for the claim that *Euphorbia esula* is absent from South Africa. The claim appears in the uppermost box titled 'Claim' and is based on various reasons, shown in boxes titled 'Reason' and each reason is supported by evidence. 'Objection' to the evidence and by inference, the reasons, are shown in boxes titled 'Objection'.

iii. Field survey for Euphorbia esula

A search was conducted in 2021 within approximately 9.4 hectares around the originally reported location (where access was granted), with a focus on the downstream area (Figure 3.8A). The surveyed area appeared to be disturbed, and the land manager reported regular mowing activities (Figure 3.8B). However, *Euphorbia esula* was not located, and it was concluded that it may have been removed due to the routine mowing practices or possibly mistaken for the alien species, *Euphorbia terracina* (Figure 3.8C), which is also present in the same region (iNaturalist, 2022a).

Figure 3.8: Evidence from field surveys for *Euphorbia esula*. **A.** Location where *E. esula* was reported. View from the Campbell Road Bridge over the Hennops River in Pretoria, Gauteng, South Africa; **B.** Location where *E. esula* was reported being mowed; **C.** *Euphorbia terracina* within Pretoria showing inflorescence (alien).

3.4.4. Risk analysis for *Euphorbia esula*

The risk score for *E. esula* in South Africa is high (Appendix 3.2). This risk score results from a combination of a scoring of massive for consequences and a probable scoring for the likelihood score (Kumschick et al., 2020a). Despite the absence of physical evidence of its presence in South Africa, its management feasibility is considered medium due to accessibility challenges and the difficulty of mechanical or chemical removal. Considering the species' absence within the country, reclassifying it onto the future 'prohibited list' is recommended to prevent resource misallocation. This recommendation is supported by both protocol analysis and field surveys that confirm its absence.

3.5. Discussion

The protocol was applied to both *C. vulgaris* and *E. esula*, resulting in a medium probability of absence for *C. vulgaris* and a high probability of absence for *E. esula*. When both species were searched for after being run through the protocol to assess the accuracy of the protocol as well as the historical records and presence status of the case study species, *E. esula* was likely to be absent as it was not found, affirming the utility of the protocol. The protocol's effectiveness in supporting informed decision-making is exemplified by the discovery of *C. vulgaris*, despite the medium probability score. While an ideal scenario would have featured a low probability score for *C. vulgaris*, the protocol's outcome indicates a medium probability. This underscores the nuanced nature of the 'medium' category, where the certainty of the species' presence or absence is less pronounced compared to the 'high' or 'low' categories, indicating a level of uncertainty that necessitates further investigation or monitoring. It is imperative to acknowledging this result as it supports the argument that the protocol works effectively when probability scores are either low or high. In cases of low probability, the protocol triggers proactive search initiatives, while in instances of high probability, it indicates that the species is likely absent, mitigating the need for extensive monitoring. The protocol also suggests that if the probability score is medium, consistent monitoring becomes imperative. This is because a medium probability indicates a level of uncertainty, suggesting the species could be present and necessitating ongoing surveillance efforts. In contrast, if the probability score is either low or high, monitoring efforts may not be as critical. A low probability score implies a higher likelihood of the species' presence, prompting proactive search initiatives, while a high probability score suggests the species is likely absent, alleviating the need for extensive monitoring.

Furthermore, the field surveys raised uncertainties regarding the historical records, specifically surrounding the original pathways of introduction. Possible intentional planting or introduction through hiking-related activities, including transport by a hiker, is considered *for C. vulgaris*, especially in scenarios where the plant may have originated in a garden adjacent to Table Mountain National Park (Ansong & Pickering, 2014; Faulkner et al., 2016; Jalal et al., 1982) however this could not be confirmed. *Euphorbia esula*'s only report in 2006, made by a citizen scientist from another country, posed challenges in validating its introduction and thus presence. All other historical evidence supported the argument for *E. esula*'s absence due to landscape changes, insufficient evidence, and a 15-year absence. These findings emphasise the critical role of reliable and accessible information, the utilisation of assessments like risk analysis, ongoing monitoring and involving stakeholders in informed decision-making for effective conservation and management of alien species.

The risk analysis examined potential impacts if *C. vulgaris* and *E. esula* were present, resulting in a high risk associated with their potential for rapid expansion and competitive displacement of native species. Despite the limited number of observed occurrences in South Africa for either species, *C. vulgaris* should be classified as Category 1a with consideration for the eradication of the solitary record, while *E. esula* should be included on a prohibited list. Accurate scoring is crucial to prevent resource misallocation, especially for species like *E. esula*, as both the protocol and field investigations support its absence within the country. Furthermore, the high likelihood of absence and high risk scores for *E. esula* suggest the need for proactive measures to prevent future introductions, including ongoing monitoring of potential pathways like agricultural seed mixtures. In summary, this evaluation underscores the significance of robust data collection, continuous monitoring, and collaboration with stakeholders to make well-informed decisions regarding the presence or absence of reported alien species. These measures are necessary for effective conservation and management strategies and the preservation of the country's native biodiversity.

While the protocol introduces a systematic approach to assess species presence or absence, it is not without limitations. There are inherent biases in the evaluation process that might persist despite the protocol's use of argument maps. However, the protocol's strength lies in its need for accurate and consolidated record-keeping, emphasising the value of centralised data. Therefore, the study also underscores the persistent challenge of inadequate data (Van der Colff et al, 2023), stressing the importance of accurate data collection and storage of records but also of search efforts. Notably, the lack of comprehensive records emerged as a significant constraint, directly affecting evidence quality and certainty in absence determinations. Despite these limitations, the study demonstrates the usefulness of the protocol, but a cautious approach is required for its broader use due to its context-dependent nature. The protocol's confirmed effectiveness serves as a foundation, but its adaptability and ability to address unforeseen challenges should be highlighted. To improve its reliability and applicability, it should be tested on more mobile taxa and under various ecological contexts and scenarios and compared with other cases of presence or absence studies made anywhere in the world.

3.6. Conclusion

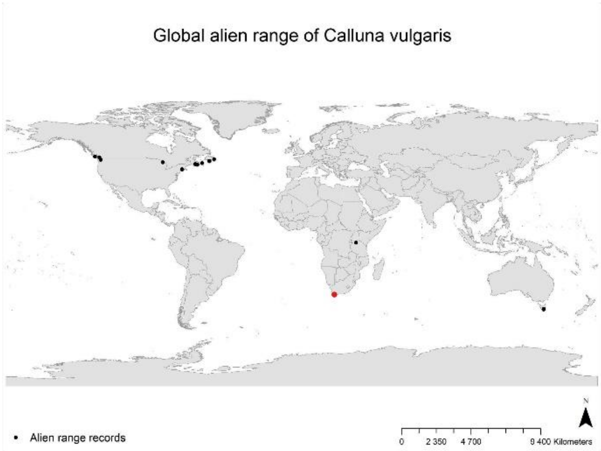
The use of the historical records along with the protocol's application and subsequent accuracy evaluation provided valuable new information about the likely presence or absence of *C. vulgaris* and *E. esula* in South Africa. The estimated probabilities of absence serve as a reminder of how difficult it is to assess such complicated conclusions. In order to properly address any possible ecological concerns associated with invasive species, this study emphasises the need for rigorous data gathering, in-depth analysis of species interactions and ecosystem impacts, and the creation of appropriate management techniques to mitigate potential harm. By producing reasonably trustworthy probabilities of absence, this methodology improves methods of inference. The protocol's success can be attributed to its systematic integration of historical data, structured information gathering, and expert judgment, which collectively provide a holistic perspective on the presence or absence of the case study species. This multifaceted approach ensures a comprehensive consideration of various factors that influence the likelihood of absence, leading to assessments that are more accurate, transparent, and robust compared to single-factor analyses. The accurate estimation of absence probabilities emphasises the intricate nature of evaluating complex events such as species presence. Furthermore, the consistency and correctness of expert judgment regarding absences could be significantly improved by argument maps and structured information gathering approaches because they force experts to be clearer about the many factors that affect the probability of absence. These tools encourage experts to state the various factors affecting absence probabilities, enhancing decision clarity and transparency. They also establish objective probability estimates, enabling discussions about uncertainty and disputes regarding species absence, with room for ongoing updates as new information emerges. This study contributes to the expanding research on alien species listings and provides a foundational reference for future investigations involving *C. vulgaris, E. esula*, and other contested alien species within South Africa and globally (Duncan et al., 2004; Anderson et al., 2007). Lastly, while the protocol has proven successful in providing a holistic perspective on the presence or absence of the case study species, there is an emerging argument for the reinstatement of a prohibited list. The removal of a single individual from its reported and confirmed location in South Africa to eradicate *C. vulgaris*, leading to a shift from Category 1a, highlights the need for a mechanism to delist species that are no longer present. The absence of a prohibited list complicates the management of such cases, necessitating a reconsideration of its role. Reintroducing a prohibited list would offer a structured approach to species management, providing clarity on the presence or absence status, and facilitating ongoing updates as new information emerges

3.7. Acknowledgements

Thank you to Kirk Bevie, Dylan Mentor, Angelique van der Berg, Thulisile Jaca and for field assistance, Dr Ashlyn Levadia Padayachee for the assistance with the risk analysis and Dr. Chad Chaney, Sishan Ebrahim and Ayesha Davids for the TMNP fire records. Ernita van Wyk is thanked for sharing information on the case study species. The South African Department of Forestry, Fisheries and the Environment (DFFE) are thanked for funding noting that this publication does not necessarily represent the views or opinions of DFFE or its employees.

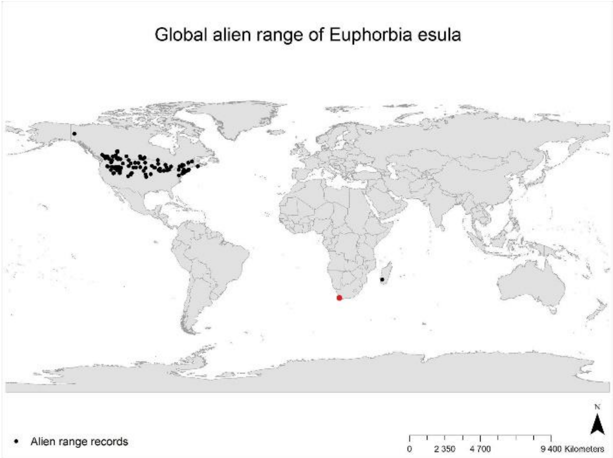
3.8. Appendices

Appendix 3.1: Summary of Risk Analysis for *Calluna vulgaris.*

Taxon: Calluna vulgaris	***Area: South Africa***
Compiled by: Chelsey Matthys	**Approved by:**
Picture of *Taxon*  Photo: Matthys, 2022	**Alien distribution map**  Reference: GBIF.org (24 October 2022) GBIF Occurrence Download https://doi.org/10.15468/dl.nv9p5f

Risk Assessment summary: *Calluna vulgaris* is present in South Africa. It is unlikely that new individuals will be brought in through unaided or human-aided primary pathways. There are, however, suitable climate and habitats within South Africa for *C. vulgaris* to thrive. It is unlikely that *C. vulgaris* will spread naturally from a population in South Africa, either with or without help from humans, to areas and habitats that are currently empty. It has the potential to invade and replace entire plant groups in its alien range by hindering plant regeneration and succession and preventing their establishment. After fire, it may dominate, and this is a risk where it is currently present within a fire-driven ecosystem in South Africa. It may have a major socio-economic and environmental impact.	**Risk score:** **High**
Management summary: The individual on Table Mountain can be removed manually by hand-pulling. It is a shrub with evergreen leaves that blooms purple flowers during a certain time of year and has high propagule persistence therefore detectability may be time-dependent. Its average lifespan is around 25 years, but it can live up to 45 years. *Calluna vulgaris* can produce seeds within two years under favourable conditions. The plant has a high reproductive capacity and may produce numerous tiny seeds that can last up to 150 years in the soil seed bank. Vegetative proliferation also occurs through rooting of drooping branches. Herbicides such as Tordon Brushkiller and Roundup may be effective in eradication attempts, but continuous weeding by hand is also an option. Dominated habitats are mostly managed through controlled burning, but recent research suggests that burning may harm ecosystem services. Eradication is feasible, given the limited presence.	**Ease of management:** **Difficult**
Recommendations: Since *Calluna vulgaris* is already in the country, it should be put on the suspect list and put in Category 1b. In the future, it should be upgraded to Category 1a following a thorough analysis of the cost and likelihood of eradication success to enforce the regulations that immediate steps must be taken to combat or eradicate the species if found in the country. Additionally, it should be included in a national management plan.	**Listing under NEM:BA A&IS lists of 2020:** **Not listed** **Recommended listing category: 1a**

Appendix 3.2: Summary of Risk Analysis for *Euphorbia esula*.

Taxon: Euphorbia esula	***Area: South Africa***
Compiled by: Chelsey Matthys	**Approved by:**
Picture of *Taxon*  Photo: *Euphorbia esula-Botanisk* Have, Aarhus C, Denmark, 09 July 2020 ID: 1467320 Egon Krogsgaard© https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:346448-1/images	**Alien distribution map**  Reference: GBIF.org (24 October 2022) GBIF Occurrence Download https://doi.org/10.15468/dl.qcg3yd

Risk Assessment summary: *Euphorbia esula,* commonly known as leafy spurge, is a non-native plant that has been reported at only one location within South Africa. Despite the report, no physical specimen has been found, and there is no evidence of its establishment or invasiveness in the country. It is a potentially invasive species that has been introduced in other regions. The plant was introduced as an ornamental in other countries within its alien range, but there is no record of it being sought after or utilised for such purposes in South Africa. It can disperse seeds through various means, including animals, birds, insects, and water. The plant's presence could have negative impacts, replacing native species, and causing economic losses in grazing areas. Preventive measures are feasible, and its intentional introduction is prohibited due to its invasive status. Detection is more successful when the plant is in flower. *E. esula* reproduces both vegetatively and via seed, making eradication challenging.	**Risk score:** **High**
Management summary: Although there is no proof of *E. esula* being in South Africa, it is essential to monitor and prevent further introductions to avoid potential spread. Management strategies focus on prevention and eradication, with strict regulations prohibiting trade or planting of the plant due to its Category 1a invasive status. *Euphorbia esula* reproduces through seeds and vegetative means, making eradication challenging due to its extensive root system. Controlled burning can be used in combination with other control methods, but it may increase plant density and sprouting if used alone.	**Ease of management:** **Medium**
Recommendations: *Euphorbia esula* should remain in Category 1a to maintain the regulations that immediate steps must be taken to combat or eradicate the species if found in the country and be put on the suspect list or Prohibited list pending an evaluation of climate suitability.	**Listing under NEM:BA A&IS lists of 2020:** **1a** **Recommended listing category:** **Prohibited list**

4. Conclusion and recommendations

A protocol designed to declare an alien species absent from a country is proposed in Chapter two, addressing concerns raised by Wilson et al. (2018a), regarding the need for clear evidence in absence declarations. The first aim for this study was thus achieved. The study's first and second aims, as outlined in Chapter one, focus on identifying listed alien species with disputed presence and developing a standardised absence declaration protocol. Chapter two delves into complexities such as identification certainty, evidence quality, temporal evidence accumulation, population loss likelihood, search effort rigor, and ongoing introductions, reshaping our understanding of species persistence. The protocol includes species details, assessment criteria, rationale, and a probability scoring system.

The protocol was applied to a marine species (i.e. *Tetraphygus niger*), solidifying its status as a tool that integrates various components. The proposed protocol, encompassed species information, assessment data, well-founded rationale, and a probability scoring system. It is useful for addressing uncertainties in invasive species presence, improving management strategies, and promoting evidence-based conservation. This ensures transparency and reliability in decision-making processes regarding species absence declarations. It also outlines details concerning the assessment process specifically the rationale behind conclusions drawn. The inclusion of a probability of absence scoring system adds a quantifiable measure of confidence to absence declarations. The protocol, using argument maps (Thompson et al., 2017), analyses species presence or absence probabilities by considering factors like identification certainty, evidence quality, timeline context, and ongoing introductions, thus enabling well-informed decisions by stakeholders (Stokes et al., 2006).

The study emphasises the protocol's usefulness through absence probability calculations, acknowledging inherent limitations and context-specific dependencies. Factors like identification certainty for similar-looking species influence absence probabilities, highlighting ecological assessment uncertainty (Marble & Brown, 2021; Verloove, 2010). Recognising these challenges, the study balanced thoroughness with practical survey constraints. Overall, the comprehensive protocol not only advances the determination of species presence or absence but also offers a structured and transparent approach for decision-making. This is particularly valuable when resource-intensive surveys are not feasible (Kotse et al., 2020; Sepulveda et al., 2012).

Aim three tests the protocol's usefulness and accuracy in Chapter three by applying it to two alien plant species, *C. vulgaris* and *E. esula*, in South Africa. Argument maps, combining reasons, evidence, and objections are employed to gain insights into the complexities of species assessment in ecological contexts (Thompson et al., 2017). The protocol's efficacy was confirmed through field observations, validating its accuracy in assessing species presence or absence. This validation was supported by confirming *C. vulgaris'* presence and *E. esula's* absence. Utilising the protocol, predictions were made and subsequent searches for both *E. esula* and *C. vulgaris*. The protocol performed well for *E. esula*, providing accurate insights into its absence based on landscape changes, insufficient evidence, and a 15-year absence. In the case of *C. vulgaris*, the protocol demonstrated its effectiveness, after the successful discovery of one individual. This favourable outcome highlights the practical usefulness of the protocol in practical applications in real-world scenarios, demonstrating the strengths, weaknesses, and nuances of species presence or absence assessments.

Furthermore, it emphasises transparency in decision-making, enabling well-informed declarations and enhanced resource allocation for management efforts. This study's implications extend to alien species management, offering insights for informed decision-making.

This research may also contribute to shaping alien species management practices by offering a systematic approach for addressing invasive alien species challenges and promoting evidence-based conservation. While the study provides recommendations for managing *C. vulgaris* and *E. esula* in South Africa, it's important to note that certain aspects, such as analysis of search and survey methods, were not included in the dissertation. While this study sheds light on the protocol's potential, there's room to enhance its effectiveness by incorporating an analysis of search and survey methods. Additionally, integrating such an analysis could enhance the study's conclusions and recommendations, refining the protocol's accuracy and enhancing tailored management measures. Stakeholders can utilise these insights to enhance species evaluation techniques and management practices.

Regular reassessments of species presence or absence are necessary to adapt to evolving taxonomic understanding and scientific knowledge, ensuring the protocol's reliability and accuracy in conservation decisions. For future research, extending the case study approach from Chapter three is essential for refining the protocol. This can be achieved by incorporating additional case studies covering a broader range of alien species and ecological contexts. Conducting field surveys in various regions with different environmental conditions and species compositions would provide valuable data to further test and validate the protocol's effectiveness across diverse ecosystems. Moreover, integrating long-term monitoring efforts to track species dynamics and responses to management interventions would offer insights into the protocol's robustness over time. Additionally, collaborating with stakeholders and experts in the field to gather their perspectives and feedback on the protocol's application and usability would contribute to its refinement and optimisation for practical implementation.

Extending this proposed protocol to evaluate the presence or absence of other species will create a versatile and adaptable protocol for diverse ecosystems and scenarios, leveraging insights from specific case studies to enhance its overall applicability (Kumschick et al., 2017). Expanding upon the protocol's application to different types of species beyond the two plant species examined in this study introduces intriguing challenges and opportunities. For instance, applying the protocol to animal species may require adjustments to account for differences in behaviour, habitat preferences, and dispersal mechanisms. Additionally, incorporating species with varying life histories and ecological roles would provide insights into the protocol's versatility and effectiveness across different taxonomic groups. Moreover, exploring the protocol's application in different geographical regions with distinct environmental conditions and management priorities would enhance its practical utility. These endeavours would enrich the understanding of species presence or absence dynamics and contribute to the development of more robust and adaptable management strategies for invasive alien species. While the case study approach proved valuable for plants, which are sessile, the dynamics change when dealing with more mobile species or those that frequently change habitats (Issaris et al., 2012; Rohde et al., 2017). For instance, birds might exhibit seasonal migrations, changing their presence within a region over time. This mobility adds complexity to assessing their presence or absence, necessitating innovative approaches (Cohen et al., 2002; Guillera-Arroita et al., 2014; Kery, 2002).It must also be noted that the protocol does primarily benefit cases where species' presence or absence is uncertain or disputed, making it a targeted tool rather than a universal solution. To enhance the efficacy of invasive species management, it is imperative to tailor management plans to the specific characteristics and threats posed by each species (Wittenberg & Cock, 2005).Overall, the protocol can enhance its own utility by incorporating feedback from users and stakeholders who apply it in different contexts. By gathering insights from practical applications and refining its methodologies based on real-world scenarios, the protocol can evolve to become more effective and adaptable. Additionally, ongoing research and development efforts aimed at expanding its scope, improving its accuracy, and addressing emerging challenges can further enhance its utility over time.

One fundamental aspect that warrants further attention is the issue of data on search effort. Often, there is insufficient information detailing the extent and methodology of search efforts aimed at detecting species within a particular region (Probert et al., 2022). This deficiency in data on search efforts can significantly impact the reliability and comprehensiveness of species presence or absence assessments. This was one of the challenges faced when developing this protocol as there was a lack of information available around hours spent searching, numbers of people who searched, and exact method of search efforts. Therefore, addressing this issue is essential to ensure more accurate and robust evaluations of species distributions and occurrences. Developing online databases of search effort could be a promising step. These databases could record when, where, and how searches were conducted, along with the results. Additionally, leveraging platforms like GBIF, where users document species observations, can provide valuable insights. Instances where species are documented as absent in GBIF records could suggest that people were present and actively searching for the species but did not encounter it. These records, if carefully curated and analysed, may provide indirect evidence of the species' absence within a specific area or region. However, it is essential to consider potential biases in reporting and recording, as well as the limitations of citizen science data, when interpreting such records.

In the context of the absence declaration protocol outlined in this study, continuous monitoring is particularly important. Long-term research serves as an essential component to evaluate the protocol's effectiveness in guiding management strategies for invasive species. Specifically, it allows for the assessment of how well the protocol performs in addressing specific aspects of invasive species management, such as monitoring and control efforts. This includes evaluating the protocol's ability to accurately identify areas where invasive species are present or absent, as well as its effectiveness in informing management decisions aimed at mitigating the impacts of invasive species over time.

In conclusion, this research has made significant strides in improving the precision, transparency, and uniformity of determinations concerning the presence or absence of alien species. Through meticulous examination and empirical testing in specific case studies, this study has effectively showcased the real-world applicability and efficiency of the developed protocol.

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