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An expert-driven framework for applying eDNA tools to improve biosecurity in the Antarctic

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Abstract

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Review

An expert-driven framework for applying eDNA tools to improve biosecurity in the Antarctic

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Abstract

Signatories to the Antarctic Treaty System's Environmental Protocol are committed to preventing incursions of non-native species into Antarctica, but systematic surveillance is rare. Environmental DNA (eDNA) methods provide new opportunities for enhancing detection of non-native species and biosecurity monitoring. To be effective for Antarctic biosecurity, eDNA tests must have appropriate sensitivity and specificity to distinguish non-native from native Antarctic species, and be fit-for-purpose. This requires knowledge of the priority risk species or taxonomic groups for which eDNA surveillance will be informative, validated eDNA assays for those species or groups, and reference DNA sequences for both target non-native and related native Antarctic species. Here, we used an expert elicitation process and decision-by-consensus approach to identify and assess priority biosecurity risks for the Australian Antarctic Program (AAP) in East Antarctica, including identifying high priority non-native species and their potential transport pathways. We determined that the priority targets for biosecurity monitoring were not individual species, but rather broader taxonomic groups such as mussels (*Mytilus* species), tunicates (Ascidiacea), springtails (Collembola), and grasses (Poaceae). These groups each include multiple species with high risks of introduction to and/or establishment in Antarctica. The most appropriate eDNA methods for the AAP must be capable of detecting a range of species within these high-risk groups (e.g., eDNA metabarcoding). We conclude that the most beneficial Antarctic eDNA biosecurity applications include surveillance of marine species in nearshore environments, terrestrial invertebrates, and biofouling species on vessels visiting Antarctica. An urgent need exists to identify suitable genetic markers for detecting priority species groups, establish baseline terrestrial and marine biodiversity for Antarctic stations, and develop eDNA sampling methods for detecting biofouling organisms.

Key words: biofouling, environmental DNA, non-native species, marine, Southern Ocean, terrestrial, risk assessment

Introduction

Antarctica has been the continent least impacted by non-native species to date, but is facing increasing pressure in both marine and terrestrial ecosystems (Bergstrom 2022; Convey and Peck 2019; McCarthy et al. 2019). Natural barriers to non-native species incursions are breaking down through the combination of increased human visitation and associated increased shipping activity (through national Antarctic programs, tourism and fisheries), and regional climate warming (Bergstrom 2022; Convey and Peck 2019; Hughes et al. 2015; McCarthy et al. 2019, 2022). To date, most marine and terrestrial non-native species incursions into the Antarctic environment have occurred in the milder Antarctic Peninsula and Scotia Arc (maritime Antarctic; Hughes et al. 2015; McCarthy et al. 2019). However, non-native terrestrial plants and invertebrates have also colonised synanthropic locations such as research stations and their immediate surrounds in continental Antarctica (Bergstrom et al. 2018; Hughes et al. 2015; Pertierra et al. 2022). Under the Protocol on Environmental Protection to the Antarctic Treaty, all signatory nations have an obligation to prevent incursions of non-native species into Antarctica (ATCM 2009). However, systematic surveillance for non-native species incursions into Antarctica remains rare, both in marine and terrestrial environments.

Managing non-native species and disease has recently been identified as one of the top three most beneficial strategies for conservation of Antarctic biodiversity (Lee et al. 2022). Previous studies have assessed Antarctic biosecurity and identified a range of taxa at high risk of future introduction to Antarctica, pathways for those taxa to reach the continent, and Antarctic locations at particular risk of invasion. For example, non-native plant species have been carried to Antarctica in the footwear, clothing and luggage of visitors (especially those who had previously visited parks, rural and agricultural areas), with the risk of their establishment predicted to be highest in the Antarctic Peninsula but expected to increase at other locations with climate change (Chown et al. 2012; Huiskes et al. 2014). Importing food and other cargo to Antarctica has been shown to provide an introduction pathway for a diverse array of terrestrial invertebrates, including flies, beetles, moths, and springtails, highlighting the importance of quarantine protocols (Greenslade and Convey 2012; Houghton et al. 2016). Antarctic shipping may also serve as an introduction pathway for species through biofouling (Lewis et al. 2003). Many hull-fouling species at risk of introduction are unlikely to survive in Antarctic shallow benthic ecosystems, but four species (two marine invertebrates and two algae) show potential to become established under current conditions (Holland et al. 2021). In a horizon scanning exercise focused on the Antarctic Peninsula region, 103 species were identified as potential invasion risks within 10 years, of which 13 species were considered high risk: this list was dominated by marine

invertebrates, but also included terrestrial invertebrates and flowering plants (Hughes et al. 2020).

Biosecurity practices should be informed by biological, environmental and physical features, as well as policy drivers and performance standards. Antarctic biosecurity surveillance is largely focused on detecting new incursions. Currently-applied surveillance methods include a range of visual surveys to identify incursions, including surveillance of cargo (Greenslade and Convey 2012; Houghton et al. 2016), remotely operated vehicle (ROV) surveys and opportunistic hull inspections (e.g., Lee and Chown 2009). However, these methods can be time- and labour-intensive (e.g., Jerde et al. 2011), and are not carried out consistently between national programs and other transport operators. Detection of a non-native species along a pathway or in Antarctica (ideally confirmed, potentially with an alternative method) instigates management actions, including targeted sampling to determine the location and extent, and an action assessment including eradication or control when possible (Bergstrom et al. 2018; Hughes and Pertierra 2016; Pertierra et al. 2017b). Changes to biosecurity procedures, as well as to policies, regulations, operations and future monitoring may also be triggered (see Figure 1).

Methods to detect DNA present in environmental samples such as water or soil, known as environmental DNA (eDNA), are now enhancing opportunities for biosecurity monitoring (Bowers et al. 2021; Zaiko et al. 2018), including in polar ecosystems (van den Heuvel-Greve et al. 2021). Genetic approaches have great potential to complement other visual survey methods (e.g., field surveys, hull inspections, ROV surveys) as part of future biosecurity monitoring in the Antarctic (Figure 1). In particular, eDNA could add capacity to survey difficult-to-access and environmentally vulnerable locations while improving detectability of non-native species incursions. Genetic databases of "DNA barcodes" (Hebert et al. 2003) can be used to assign taxonomy and confirm detection of a non-native species in an eDNA sample. Detecting non-native species can inform future monitoring approaches, e.g., using a more sensitive species-specific eDNA assay, while a detection that is subsequently recognised as a false positive may require monitoring methods to be adjusted.

eDNA methods are already employed for non-native species surveillance in monitoring programs around the world (e.g., Mize et al. 2019; Trujillo-González et al. 2022; Zaiko et al. 2018) and have been used to detect non-native marine species in the Arctic (van den Heuvel-Greve et al. 2021). In the sub-Antarctic, eDNA extracted from lake sediments has been used to trace the impact of invasive rabbits on native plants over time (Ficetola et al. 2018). Cowart et al. (2018) detected eDNA from predatory king crabs (Lithodidae) in water from the western Antarctic Peninsula continental shelf, suggesting eDNA could be used to track any range expansion from the continental slope to shelf ecosystems. However, when considering the

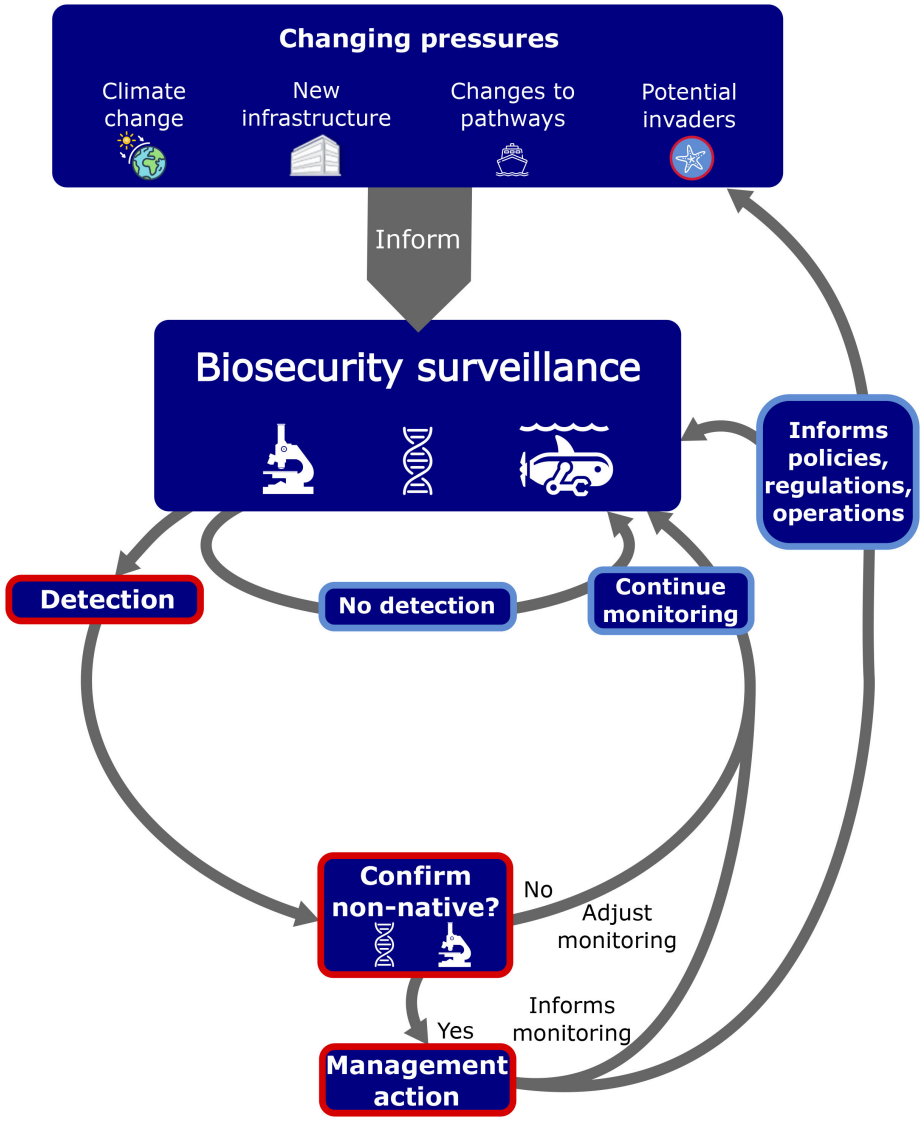

Figure 1. The potential role of environmental DNA for biosecurity and surveillance in Antarctica.

incorporation of eDNA methods into a routine biosecurity monitoring program, it is important to ensure that the sampling strategies and methods employed are fit-for-purpose and add value to existing survey methods (Bowers et al. 2021).

When applying eDNA methods to biosecurity surveillance, it is crucial to reliably distinguish non-native target species from native biodiversity. However, an important limitation here is that knowledge of Antarctic biodiversity is incomplete. For example, invertebrate communities in both marine and terrestrial Antarctic ecosystems are generally poorly characterised, and genetic studies increasingly reveal that cryptic species are common in both marine and terrestrial Antarctic ecosystems (Brunetti et al. 2021; Carapelli et al. 2020; Kaiser et al. 2013; Stevens et al. 2021; Velasco-Castrillón and Stevens 2014), and that potential invasive species may also represent species complexes (e.g., Brunetti et al. 2015; Wilson et al. 2018). Furthermore, reference DNA sequence data (e.g., DNA barcodes) are not

available for most Antarctic native species. This lack of baseline data can prevent species-level resolution (although resolution to genus or family level may be possible), and makes it difficult to ensure eDNA tests can distinguish non-native from native Antarctic species.

Ensuring that eDNA methods are appropriate for aiding Antarctic biosecurity efforts requires knowledge of priority species or taxonomic groups for which eDNA surveillance will be informative, and the availability of genetic resources for those target species or groups. This includes validating assays to detect specific species, e.g., real-time PCR, or taxonomic groups, e.g., DNA metabarcoding. Augmenting reference DNA sequence databases to ensure eDNA tests can robustly distinguish between the target species and related native Antarctic biota would facilitate development of targeted biosecurity assays. Applying targeted taxonomic effort to groups that are poorly known in Antarctica but related to potential invaders is also essential. These measures could also improve confidence in detections of invasive species during broader eDNA biodiversity monitoring, e.g., by enabling discrimination of native and non-native taxa when using "universal" eDNA metabarcoding approaches to characterise Antarctic ecological communities.

In this study, we determine priorities for developing eDNA biosecurity monitoring in Antarctica, specifically in the operational area of the Australian Antarctic Program (AAP). The AAP has recently acquired a new research vessel (RSV *Nuyina*), and several Australian Antarctic stations are undergoing modernisation and rebuilding over the next two decades, increasing the amount of shipping, aviation, personnel and, inevitably, the associated biosecurity risks. We present case studies to illustrate key considerations for applying eDNA methods to Antarctic biosecurity, including benefits and outstanding questions regarding eDNA-based approaches.

Materials and methods

Risk assessment of potential invasive non-native species

To inform the use of eDNA methods for Antarctic biosecurity, we used a modified Delphi procedure, where a panel of experts were asked over a series of workshops to identify the highest priority non-native species (or taxonomic groups), including transport pathways for their introductions, using multiple rounds of consultation in order to achieve a consensus opinion (Hemming et al. 2017). Workshop discussions were focused on introductions to the Australian Antarctic Program's operational footprint, specifically the three research stations (Casey, Davis and Mawson, Figure 2, Supplementary material Figure S1) in Antarctic Conservation Biogeographic Regions (ACBR) No. 7, East Antarctica, and No. 16, Prince Charles Mountains (Terauds and Lee 2016). Previous risk assessments for non-native species introductions to Antarctica have focused on a restricted range

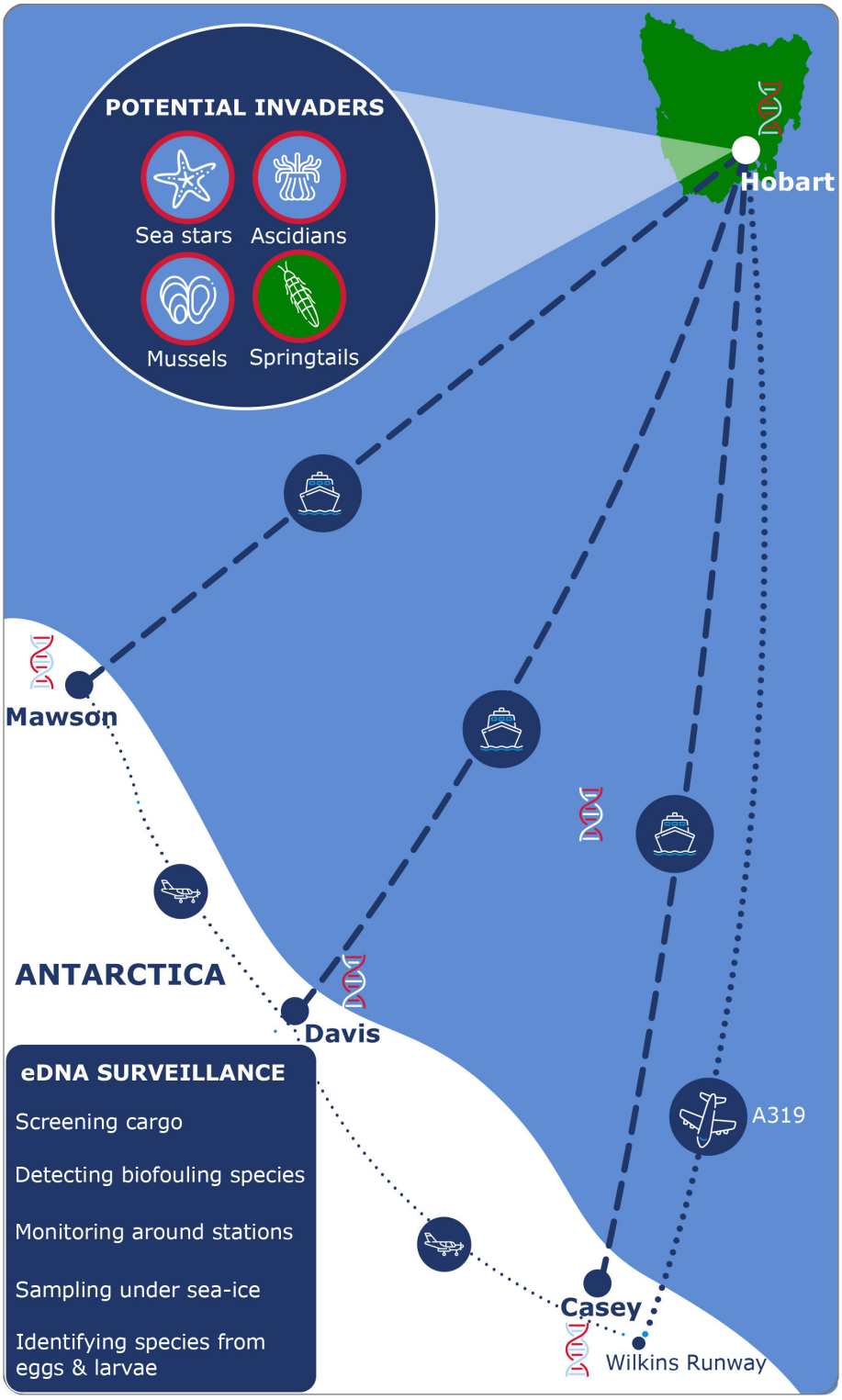

Figure 2. How environmental DNA can contribute to biosecurity surveillance in the Australian Antarctic Program. The figure shows some of the potential invaders, their Australian source populations (Tasmania/Derwent estuary), pathways (shipping and flights), some of the locations where eDNA can be sampled (cargo, ships, stations, under sea-ice), and some benefits of incorporating an eDNA-based approach (e.g., sampling under sea-ice, and identifying species from eggs and larvae).

of taxa (Chown et al. 2012; Greenslade and Convey 2012; Houghton et al. 2016; Huiskes et al. 2014; Pertierra et al. 2017a, 2020; Vega et al. 2021), or on specific transport pathways (e.g., hull-fouling, Holland et al. 2021), or at

locations with limited relevance to the Australian Antarctic Program (e.g., Antarctic Peninsula, Hughes et al. 2020). Expert elicitation had the dual aims of undertaking a risk assessment and identifying priority species for future development of eDNA surveillance, focusing on known taxonomic entities, consideration of pathways, and known terrestrial and marine Antarctic habitats.

Project scope

We explored the risk of non-native species (excluding microorganisms) being introduced to and establishing in both terrestrial and marine environments in the vicinity of the three Australian Antarctic stations. Freshwater species were considered lower risk due to the lack of a clear pathway for their introduction (Hughes and Convey 2012), and were therefore not included in this study. The primary departure point for shipping and flights to the Australian Antarctic stations is Hobart, situated on the Derwent Estuary, Tasmania (Figure 2), and risks from this location framed our discussions. Other less-utilised departure points (e.g., Fremantle, Western Australia), as well as ports visited by Australian Antarctic supply vessels prior to their departure from Hobart, were also acknowledged. Non-Australian vessels also very occasionally visit Casey, Davis and Mawson. National Antarctic programs of all Consultative Parties to the Antarctic Treaty are required to follow the Protocol on Environmental Protection to the Antarctic Treaty (Annex II, ATCM 2009), which stipulates measures to reduce the risk of non-native species incursions. However, the AAP does not oversee biosecurity measures taken by other national programs, so possible introductions via vessels from other Antarctic programs were not considered directly in this risk assessment. Intracontinental flights visit the Casey (Wilkins aerodrome and skiway) and Davis (Vestfold Hills) regions and have the potential to transport propagules from the Northern Hemisphere, through South America and the Antarctic Peninsula to East Antarctica (cf. Hughes et al. 2019). Helicopters with expeditioners from nations with research stations in the Larsemann Hills (100 km to the east of Davis station) also conduct occasional visits to Davis Station and the surrounding Vestfold Hills in most years (pre-COVID-19 pandemic). No tourism operators currently visit the Australian Antarctic research stations, so tourist vessels were also not considered in this study, although they could pose a risk in the future. Tourist ships regularly visit sub-Antarctic Macquarie Island (where the AAP also operates a year-round station) during the summer months, but biosecurity risks to Macquarie Island were beyond the scope of this study.

As our focus was on species not native to Antarctica, our exploration of intra-regional transfer of native species within Antarctica was minimal, despite their potentially high risk of introduction and establishment (Hughes et al. 2019). We did consider the risk of transfer from sub-Antarctic

Macquarie Island to Casey, Davis, and Mawson in Antarctica but, given current shipping routes (ships typically visit continental stations prior to rather than after Macquarie Island), the risk was deemed low. However, should the sequence of station visits change in the future, biosecurity considerations for the AAP should be reviewed.

Compiling a preliminary list of potential invasive non-native species

A preliminary list of species with potential to arrive and establish in Casey, Davis, Mawson and surrounds was created based on published literature. Species included: high risk non-native species threatening the Antarctic Peninsula (Greenslade and Convey 2012; Hughes et al. 2020); marine (or brackish) species on the Global Invasive Species Database with distributions including sub-freezing temperatures (Holland et al. 2021); invasive species in the Derwent Estuary (Whitehead 2008); Derwent Estuary species that could survive Antarctic temperatures (Lewis 2007); non-native marine species previously recorded in Antarctica (though none are known to be established; Laeseke et al. 2021; McCarthy et al. 2019); non-native terrestrial species that have become established in Antarctica at least once (Enríquez et al. 2019; Hughes et al. 2015); invertebrates that have colonised station buildings and/or wastewater treatment plants at Antarctic stations (Hughes et al. 2015); and seeds/propagules from non-native plant species previously identified in Antarctic visitor samples (e.g., in clothing) that occur in the Arctic and/or sub-Antarctic (Chown et al. 2012). Taxa that could not be resolved to at least genus level (e.g., "unidentified mosquito"), were excluded from further consideration.

Expert evaluation of preliminary species list

Panel members (n = 26) were invited to contribute to the risk assessment based on their expertise in one or more areas of Antarctic or sub-Antarctic invasive species ecology and invasion pathways, Antarctic ecosystems, and/or Australian/New Zealand invasive species. The expertise of panel members covered a broad range of relevant taxonomic groups. Each panel member was asked to review the preliminary list of potential invasive non-native species and to independently score, for each species within their area of expertise: (a) the risk of arrival at Australian Antarctic research stations in East Antarctica (Casey, Davis or Mawson) and surrounds; (b) the risk of establishment in the natural environment (i.e., beyond immediate station environs); and (c) the magnitude of the species' potential negative impact on Antarctic ecosystems, including their biodiversity. A five-point scale was used for scoring in each category as per Hughes et al. (2020), where 1 = very low risk and 5 = very high risk. Participants were also asked to suggest additional species they thought should be considered as part of the prioritisation process.

Compiling a ranked shortlist of high-priority species

Following individual evaluations of the preliminary species list, a series of online workshops were held to consider species in more detail within three broad groups: marine species (no. panel members = 16), terrestrial invertebrates (n = 10) and terrestrial plants (n = 9). In each workshop, the median of participant scores for each species within the relevant group were presented and used as a basis for discussion. Potential introduction pathways and knowledge gaps impeding scoring (e.g., taxonomic uncertainty, lack of knowledge of physiological tolerances with respect to Antarctic conditions, lack of knowledge of potential impacts of species on Antarctic ecosystems) were also discussed.

Based on the initial scores and expert opinions, a shortlist of high priority species was created from the preliminary species list within each broad group. We required that each species on the shortlist had an identified pathway of arrival to continental Australian Antarctic research stations. In addition, the shortlisted high priority species were chosen to ensure inclusion of at least one representative for each taxonomic group of interest, as well as one representative for each transport pathway. The number of species chosen for each taxonomic group reflected, to some degree, the expertise of the participants. For example, the terrestrial invertebrate group included several experts who had published research on springtails (Collembola), and these experts proposed additional springtail species and attributed high-risk scores to them.

For each species included in the shortlist, we aimed to derive consensus scores among the experts for arrival, establishment and impact. Confidence levels for each of these scores (low, medium or high) based on the evidence available for each species were also sought, and this helped to identify knowledge gaps. During the workshops, however, the difficulty of scoring the potential impact of non-native species in the continental Antarctic environment—which has no equivalent environment elsewhere on Earth—became evident. For example, a non-native species that is invasive in a temperate or sub-Antarctic environment would not necessarily be invasive in the continental Antarctic environment. For this reason, attributing realistic, robust, and defendable scores to "impact" was deemed too difficult by the majority of experts, hence low confidence was attributed to "impact" scores more often than the "arrival" or "establishment" scores (Tables S1–S3).

Following the workshop discussions to generate priority lists, participants were asked to review scores and rankings and, if necessary, re-score as appropriate to give a final ranked list for each of the three broad groups.

We used the combined risk scores (combined score = Arrival × Establishment × Impact), to generate ranked lists for each of the three broad groups separately (i.e., marine, terrestrial plants and invertebrates).

The combined scores were used to create a cut-off for excluding low-risk species from the final ranked list and are not reported here. Ideally, a single scoring system would be applied to terrestrial and marine species for the risk assessment, allowing comparison of combined risk scores between species and habitats, as has been done for the Antarctic Peninsula by Hughes et al. (2020). However, this was beyond the scope of this project and the experts' capacity.

Genetic resources for high-priority species

We reviewed the available genetic resources relevant to targeted eDNA detection for each of the shortlisted high-priority species, including: species-specific PCR assays; DNA barcode sequence(s) that serve as a reference for assigning taxonomy to eDNA sequences; and mitochondrial or chloroplast genomes that are typically the source of DNA barcodes for animals and plants, respectively. The availability of species-specific assays (e.g., quantitative PCR or digital droplet PCR) was determined via literature and database searches (e.g., https://www.marinepests.gov.au/what-we-do/research/compendium-marine-pest-studies). DNA barcode and mitochondrial/chloroplast genome availability were determined by searching GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and the Barcode of Life Database (BOLD, Ratnasingham and Hebert 2007).

Results and discussion

Using regional distributions to inform eDNA testing

Experts highlighted that the absence of numerous taxonomic groups within native Antarctic biodiversity must be considered when applying eDNA methods to inform biosecurity in Antarctica. Many taxonomic groups that include invasive species are absent from continental Antarctica (e.g., insects, grasses and other flowering plants in terrestrial ecosystems, mussels and crabs in nearshore ecosystems, see below). The regional distributions of species must also be considered; for example, two flowering plants are native to the Antarctic Peninsula and springtails (class Collembola) are native to parts of continental Antarctica, yet these groups are not recorded from the regions surrounding Australian Antarctic stations. This simplifies eDNA testing for these groups at these locations, as detecting DNA from any species within such groups would warrant further investigation.

High-priority species

Based on expert opinion and consensus approaches, we short-listed 43 high-priority candidate non-native species (14 marine, 19 terrestrial invertebrates, 10 terrestrial plants, Tables S1–S3) with the highest risk of introduction and/or establishment through the AAP. The shortlist included seven of the 13 highest risk species identified as threatening the

Antarctic Peninsula (6/7 marine species, 1/7 terrestrial invertebrates, Hughes et al. 2020). Many of the short-listed species are also high risk for the Antarctic Peninsula despite distinct source populations and pathways (Americas vs. Australia), due to the wide global distributions of many invasive species in temperate ecosystems, especially in Antarctic gateway cities and adjacent nearshore environments. Short-listed species and taxonomic groups included those present in Tasmania, with a high likelihood of arrival (e.g., hull-fouling species), and/or non-native species that have previously established in the Antarctic Peninsula or sub-Antarctic region. Some of the short-listed species are known to be capable of persisting in extreme environments, as encountered in East Antarctica (Tables S1–S3), but the tolerance of other species to e.g., sub-zero temperatures is less well understood. This is a recognised data gap: in a review of life history traits of marine invasive species, Byers et al. (2015) report missing data on temperature tolerance for more than 25% of the 138 species they considered. Locations such as station buildings and infrastructure provide microhabitats with less extreme environmental conditions, and have previously been shown to support non-native species that could not persist elsewhere on the continent (Hughes et al. 2015). Detection and mitigation against incursions of non-native species into these anthropogenically-modified environments in Antarctica are also required under the Protocol on Environmental Protection to the Antarctic Treaty (Annex II, Article 4).

Marine species

A total of 14 marine species were shortlisted (Table S1). These species are taxonomically diverse and represent five animal phyla as well as green and brown seaweeds. Eleven of the species were already recognised as invasive elsewhere, and 12 were present in the Derwent Estuary. Many of the species are likely to be transported via ship biofouling, however, the larvae of some species may also be transported via ballast water.

The highest ranked marine species was the invasive ascidian (tunicate) *Styela clava*, with two other ascidians also included on the shortlist. eDNA tests targeting non-native ascidians would need to distinguish between these three and several native ascidian species that occur in the Casey and Davis nearshore environment. Two of the top three ranked marine species were bivalve mussels (*Mytilus* spp.). There are no native *Mytilus* species in Antarctica. However, recent transportation and settlement of *Mytilus* spp. (but not establishment) in the South Shetland Islands (Cárdenas et al. 2020), and their detection on Antarctic supply vessels (Lee and Chown 2007) highlights their risk of introduction and establishment in Antarctica.

Terrestrial invertebrates

The terrestrial invertebrate shortlist comprised 19 species, including nine springtails, six insects, and two mites (Table S2). Many of these shortlisted

species are known invasive species, having established in the sub-Antarctic, the Antarctic Peninsula, and/or within station environs at Casey (the midge *Lycoriella* sp., Hughes et al. 2005).

Terrestrial plants

The shortlist of priority terrestrial plants included 10 species: six weedy flowering plants and four moss and liverwort (bryophyte) species (Table S3). Mosses and lichens are the dominant vegetation in continental Antarctica, and experts considered that the current climate in the Casey, Davis, and Mawson regions is likely to limit the establishment of vascular plants from the most likely source populations (Tasmania), but a changing climate could enable vascular plants to colonise in the future. Two of the highest risk plant species identified in the priority shortlist are grasses (Poaceae). *Poa annua* is invasive in the sub-Antarctic and has established in the Antarctic Peninsula (Molina-Montenegro et al. 2012; Olech and Chwedorzewska 2011). Experts considered that eDNA may have limited use for monitoring non-native terrestrial plants as visual surveys provide a simple method for detecting macroscopic vascular plants, although other genetic methods such as DNA barcoding can be used to identify seeds and other propagules, as well as non-native mosses.

Genetic resources for high-priority species

Species-specific real-time PCR (e.g., quantitative PCR or digital droplet PCR) assays already exist for all of the identified high-priority marine species (Table S4), with the exception of one crab (*Halicarcinus planatus*) and two seaweed (*Ulva*) species. Such assays are not yet available for the high-priority terrestrial invertebrate and plant species, although one is available for a close relative of the grass *Poa annua* (*P. pratensis*, which is invasive on sub-Antarctic South Georgia and was persistent at a single location on the Antarctic Peninsula until recent eradication (Pertierra et al. 2017b)), and a genus-specific assay exists for *Agrostis* species (Rowney et al. 2021). An eDNA assay for characterising multi-species assemblages (eDNA metabarcoding, Leray et al. 2013) has already been used to detect many of the identified high-priority marine invasive species in several countries (Grey et al. 2018; Holman et al. 2019; Rey et al. 2020). Reference DNA sequences are available in the form of DNA barcodes or mitochondrial/chloroplast genomes for all high-priority non-native species except for one terrestrial mite (*Coccotydaeolus* cf. *krantzii*, Table S4). However, the availability of reference sequences for native Antarctic relatives was not evaluated. If eDNA biosecurity screening is implemented in Antarctica, reference sequences will also need to be generated for relevant native Antarctic relatives to inform interpretation of eDNA data. Appropriate reference sequence databases are recognised as being crucial to the

interpretation of eDNA data for conservation and wildlife management questions (De Brauwer et al. 2023; Marques et al. 2021; Monchamp et al. 2023). Despite their importance, however, developing comprehensive reference sequence databases for all native Antarctic species will not be a trivial task because of, for example, uncertainties around the composition and taxonomy of Antarctic communities, and practical difficulties in accessing specimens suitable for reference sequencing from remote locations.

Key considerations for applying eDNA to Antarctic biosecurity

In this study, we generated shortlists of high-priority candidate non-native species with the highest risk of introduction and/or establishment to Antarctica via Australian Antarctic Program transport pathways (Tables S1–S3). Although other species and groups may pose a similar threat to the ecosystem, the proposed candidates represent clear threats and require monitoring. We combined these lists with knowledge regarding the availability of genetic resources for these high-priority species (Table S4) to provide the basis for developing eDNA biosecurity monitoring in East Antarctica. Below, we provide illustrative case studies of how eDNA could be applied to Antarctic biosecurity with particular reference to the AAP in order to illustrate key considerations, benefits, outstanding questions and example management actions that should follow a positive eDNA-based detection of a non-native species.

1) Biosecurity surveillance for nearshore Antarctic ecosystems

The taxonomic diversity of the short-listed candidate species highlights that Antarctic eDNA biosecurity surveillance should be co-designed and implemented alongside native biodiversity surveys in a joint eDNA monitoring framework, given the potential overlap in sampling and analysis methods. Large components of Antarctic marine invertebrate biodiversity are taxonomically unknown, or untested with modern methods, and this knowledge gap creates a substantial obstruction to non-native species detection and subsequent management actions. Unlike typical national biosecurity surveillance programs that target a smaller number of defined species, Antarctic biosecurity surveillance should focus on broader taxonomic groups to maximise the potential to detect incursions. eDNA methods capable of detecting a broad range of species within high-risk taxonomic groups, such as eDNA metabarcoding, have the added advantage of informing managers on the native biodiversity present. Formal monitoring programs for terrestrial and marine Antarctic biodiversity need to be implemented, and eDNA methods should be incorporated into monitoring programs to detect community changes.

eDNA metabarcoding could be used to screen samples from nearshore sites close to coastal Antarctic research stations for the presence of non-

native marine taxa. Metabarcoding uses high-throughput sequencing of taxonomically-informative DNA barcoding genes to identify the taxa present in a mixed sample, and is best suited to surveying multiple species within a given taxonomic group (e.g., fish, plants, insects). A metabarcoding assay for marine metazoans (Leray et al. 2013) could be used to screen for most of the high-priority marine species highlighted in this study except for the seaweeds, albeit with the caveat that DNA from abundant native species may prevent detecting early stages of invasion by non-native species. The same metabarcoding approach has been used to survey benthic biodiversity near Davis station (Clarke et al. 2021), highlighting the potential to co-design eDNA-based biosecurity surveillance and native biodiversity monitoring in a joint framework. Future work will be needed to confirm whether all high-priority marine species would be detected with the currently available metazoan PCR primers with sufficient sensitivity and, if not, primers targeting specific groups (such as ascidians and mussels) should be designed and validated following best-practice guidelines (De Brauwer et al. 2022b; Thalinger et al. 2021). Similarly, any eDNA surveillance program should be designed following best practice and taking key principles into account as per De Brauwer et al. (2022a). In this context, multiple sample types should be collected (e.g., filtered seawater, biofilms from settlement plates, sediment grabs, plankton tows) from sites near AAP operations (e.g., wharves, mooring sites), as different taxa are more likely to be detected in different sample types based on their biology, habitat preferences, and mode of shedding DNA into the environment (Holman et al. 2019; Koziol et al. 2019). As always, knowledge of the biology of the target organism matters when sampling (Adams et al. 2019; Andruszkiewicz et al. 2021).

In the case of an eDNA detection of a non-native species in nearshore Antarctic environments, we recommend follow-up sampling of the same location and sample type. A species-specific real-time PCR assay could then be used to provide increased sensitivity (such assays are available for almost all high-priority marine species, Table S4), combined with spatially explicit sampling designs (e.g., stratified random sampling) to narrow down the location or spread. eDNA detections could also be used to trigger detailed visual surveys (e.g., by ROV) to confirm non-native species presence, followed by rapid assessment of possible actions as per the Antarctic Treaty's Non-Native Species Manual (CEP 2019).

2) Biofouling by marine non-native species on Antarctic vessels

eDNA has the potential to enhance routine surveillance of high-risk pathways, such as biofouling on Antarctic vessels, by allowing detection of larvae or juveniles from high-priority species that are difficult to identify with other methods (Zaiko et al. 2016). We recommend applying eDNA methods to detect non-native biofouling species in samples from Antarctic

vessels. Biofouling is likely to be a higher risk than ballast water for the introduction of non-native species to Antarctica. The Antarctic Treaty Consultative Meeting and International Maritime Organisation have adopted the "Practical Guidelines for Ballast Water Exchange in Antarctic Waters", requiring ballast water exchange at the Polar Front (McCarthy et al. 2019), which should reduce the introduction of non-native species to Antarctic nearshore environments via ballast water. The International Maritime Organization Ballast Water Management Convention, which will be fully implemented by 2024, sets even higher standards for vessels from signatory nations (https://www.imo.org/en/About/Conventions/Pages/International-Convention-for-the-Control-and-Management-of-Ships%27-Ballast-Water-and-Sediments-(BWM).aspx). However, ship surfaces exposed to seawater but not ice-scour or high-water flow (e.g., the sea chest, moon pool, wet wells, drop keel, internal seawater systems) are at high-risk of biofouling by non-native species (Hughes and Ashton 2017; Lee and Chown 2009; Lewis et al. 2004). Sea chests and the associated internal seawater systems are complex, and often unique to individual vessels (Davidson et al. 2021). Nonetheless, some polar vessels re-circulate water in internal systems to maintain temperatures warmer than the surrounding seas, potentially allowing temperate marine organisms to survive and reproduce (Fletcher et al. 2017). For example, the sea chest of the RSV *Nuyina* is maintained above 14 °C. Sea strainers, which are located inside the ship, may provide a means to sample communities within internal seawater lines, including during transit. Other internal areas prone to biofouling can be sampled during transit, on departure from Australia, or on arrival in Antarctica. The timing of such sampling should be carefully considered. For example, sampling while a vessel was in dock post-season might detect invasive species eDNA that originated from local waters, rather than from biofouling organisms within the ship's system. The marine metazoan metabarcoding assay described above could also be used to analyse samples from vessels. eDNA metabarcoding assays targeting biofouling groups such as ascidians and mussels should be developed to improve sensitivity.

Positive detection of non-native species on ship surfaces would confirm presence of the taxon within the biofouling pathway to Antarctica. We recommend a positive detection of this type be confirmed by morphological identification, or DNA barcoding of tissues or whole individuals. The identity of the species may then trigger operational changes to prevent incursions, e.g., additional biofouling treatments, exclusion screens, filtration, etc. Species-specific real-time PCR assays could be used for any non-native species detected to improve sensitivity for future biosecurity monitoring. Advances in field DNA extraction and detection methods are making rapid in-field monitoring of aquatic non-native species a possibility (e.g., Jeunen et al. 2022; Thomas et al. 2020).

3) Biosecurity surveillance for terrestrial invertebrates

eDNA can be used to detect and identify eggs and larvae of terrestrial invertebrates from an environmental sample that cannot typically be identified with other methods, or even species with very small adult life stages, such as springtails (Collembola) or mites (Acari). Many springtail species are present elsewhere in continental Antarctica, but no native springtails have been found to date in the Casey, Davis or Mawson regions. Numerous non-native springtails have been introduced and established on sub-Antarctic islands, including Macquarie Island (e.g., Greenslade 1990; Phillips et al. 2017). A non-native springtail (*Xenylla* sp.) was detected and subsequently eradicated from Davis station in 2014 (Bergstrom et al. 2018), highlighting the risk of introducing Collembola via the AAP. Springtails also represent the greatest non-native invertebrate diversity in the northern Antarctic Peninsula region (particularly the South Shetland Islands, Greenslade et al. 2012; Hughes et al. 2015), highlighting that they may become a higher risk group for more southern latitudes under predicted climate warming scenarios. However, they also provide an example of a group where the greatest risk is that of intra-Antarctic transfer of species native to other climatically similar regions of the continent (cf. Hughes et al. 2019). This has important implications for the AAP as it requires consideration of the potential for intra-regional transfer between distinct Antarctic Conservation Biogeographic Regions, including the East Antarctica and Prince Charles Mountains ACBRs (Terauds and Lee 2016).

eDNA could be used to test for the presence of Collembola or other invertebrates in supplies and equipment taken to Antarctica by the AAP (e.g., food, clothing, footwear, machinery, cargo, etc.), and to monitor cargo facilities in Australia, as well as high-risk sites at the stations (e.g., hydroponics facilities, food stores, high foot-traffic sites, Madden et al. 2016; Trujillo-González et al. 2022). Such methods, if implemented, could also be used to monitor risk of intracontinental incursions of springtails (and plant propagules) via air transport from the Antarctic Peninsula and other regions. Splitting and preserving separate sub-samples where possible, prior to DNA extraction, would allow visual inspection or further molecular testing of positive samples.

Confirmed invertebrate detection along the available pathways would highlight the propagule risk, and suggest the need to enhance biosecurity actions to eliminate the source prior to transport to Antarctica. We recommend that any detection of invertebrate DNA in the field or on station should trigger visual inspection of the site and any remaining sample (if samples were split prior to eDNA analysis), followed by expanded molecular sampling. More sensitive species-specific assays should also be developed, as none are currently available for any of the priority invertebrate species.

4) Assessing non-native species management

Where a non-native species incursion has occurred, eDNA methods can provide a targeted, sensitive means to locate populations and assess management efforts. The black fungus midge (*Lycoriella* sp.) has been established at Casey station since 1998 (Hughes et al. 2005) and current understanding is that the population is reproducing in the sub-floor area of the accommodation building (A. Sharman *pers. comm.*). eDNA testing for *Lycoriella* at Casey station could be used to locate habitat or sites of larval development, and repeated sampling could be used to assess eradication actions. Sampling for *Lycoriella* DNA at Casey should target damp areas of the sub-floor soil surface, where eggs are laid and larvae develop into adults (A. Sharman *pers. comm.*). Currently no species- or genus-specific eDNA tests are available for *Lycoriella* sp., but these could be developed, which would also require generating reference DNA sequence data for this species. As no other insects are native to continental Antarctica, an insect- or fly- (order Diptera) specific test would also be suitable for use in more general eDNA surveys.

eDNA testing should be combined with traditional monitoring (e.g., light or sticky traps) to detect adult invertebrates in order to confirm species presence and numbers (Bartlett et al. 2019; Remedios-De León et al. 2021). Note that, for this and the case studies outlined above, there would be a time-lag of weeks to potentially months between sample collection and a detection using real-time PCR or metabarcoding due to the need for specialised laboratory equipment. If there were a management need for more rapid sample turnaround (e.g., to screen cargo pre-departure), we recommend developing portable molecular assays that can be used in the field (e.g., Biomeme platform, http://biomeme.com/). Environmental RNA (eRNA) assays can also help distinguish between genetic material from living or dead organisms (Merkes et al. 2014; Pochon et al. 2017).

5) Persistence and transport of eDNA in Antarctic environments

An outstanding question regarding the application of eDNA to Antarctic biosecurity is whether an eDNA detection indicates species presence at, or close to, the sampling site (Clarke et al. 2021), and over what temporal scale. In marine environments, ocean currents and tides can potentially transport eDNA several kilometres at least (Cowart et al. 2018; Ellis et al. 2022), confounding interpretation of whether the DNA came from a local or distant source. Although several studies have demonstrated that marine eDNA provides a signal of local biodiversity and captures fine-scale habitat variation (e.g., Jeunen et al. 2019; Monuki et al. 2021; West et al. 2020), eDNA persists much longer in cooler Antarctic waters (−2 to 0 °C) compared to temperate waters (Cowart et al. 2018; Ellis et al. 2022), increasing the potential to detect eDNA at greater distances from the source organism.

DNA preservation is also better in permanently cold Antarctic terrestrial environments (e.g., Fraser et al. 2018) or environments with low microbial activity (Salter 2018), with ancient DNA representing the most extreme example (e.g., Turney et al. 2020). The potential for eDNA to persist and be transported in Antarctic environments could confound interpretation of eDNA detections. Future research should seek to improve knowledge of eDNA dynamics (transport and persistence) in Antarctic marine and terrestrial environments (e.g., Ellis et al. 2022) and explore new opportunities presented by eRNA, e.g., how long do eDNA and eRNA persist in Antarctic environments and how does this influence detectability?

Conclusions

Based on our priority shortlists, eDNA monitoring, both for the AAP and in a more general Antarctic context, will provide significant benefits to biosecurity surveillance by targeting, in particular, 1) marine species in nearshore environments, 2) biofouling species on ships, and 3) terrestrial invertebrates both in transit and in Antarctica. The next steps towards implementation should ensure the availability of necessary resources and infrastructure, to enable eDNA monitoring to become a routine component of Antarctic biosecurity surveillance. Key molecular developments that are needed include: evaluating suitable genetic markers for detecting priority species groups (accompanied by improved taxonomic understanding to ensure target species can be distinguished from related native species based on reference DNA data); developing and validating new assays more specific to groups of interest (e.g., seaweeds, ascidians, mussels) where improved sensitivity is needed; and developing eDNA sampling protocols for detecting biofouling organisms on Antarctic vessels. The outcomes of this study were focused on AAP operations, but demonstrate a critical need to establish baseline terrestrial and nearshore marine biodiversity surveys and ongoing monitoring for all Antarctic research stations and their surroundings, which would employ multiple survey methods, including eDNA.

The Antarctic Treaty System's Committee for Environmental Protection has made the introduction of non-native species a Priority 1 issue in its Five-Year Work Plan, and has included the action to "*Develop a surveillance strategy for areas at high risk of non-native species establishment*". With all Antarctic Treaty signatory nations committed to the prevention of incursions of non-native species into Antarctica, the systematic application of eDNA technology and the approaches described here for biosecurity surveillance of operations, including around Antarctic stations, may help deliver this action. Working together with Antarctic operators who are developing eDNA surveillance programs outside the AAP will improve consistency of approaches across the Antarctic region. Raising awareness of the benefits of eDNA tools for biosecurity amongst Antarctic Treaty Consultative Meeting/Committee for Environmental Protection policy

makers and Antarctic operators may facilitate development of a continent-wide systematic approach to reducing the risk of non-native species entering the Antarctic environment.

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Author's contribution

A.J.M., L.J.C., L.S., D.M.B., J.D.S., J.S.S., C.K.K.: research conceptualization; A.J.M., L.J.C., J.D.S.: sample design and methodology; All authors: investigation and data collection; L.J.C.: data analysis and interpretation; L.J.C., A.J.M., D.M.B., C.K.K., J.D.S: writing – original draft; All authors: writing – review and editing.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Map of Antarctica and the Southern Ocean including year-round Australian stations. Sourced from the Australian Antarctic Data Centre (https://data.aad.gov.au/map-catalogue/map/14159) under a Creative Commons Attribution 4.0 Unported License.
Table S1. Ranked list of marine species that represent the greatest perceived risk of arrival, establishment, and impact via the Australian Antarctic Program.
Table S2. Ranked list of terrestrial invertebrate species that represent the greatest perceived risk of arrival, establishment, and impact via the Australian Antarctic Program.
Table S3. Ranked list of terrestrial plant species that represent the greatest perceived risk of arrival, establishment, and impact via the Australian Antarctic Program.
Table S4. Genetic resources currently available for priority species, including species-specific real-time PCR assays, and reference sequences for DNA barcoding genes or mitochondrial/chloroplast genomes.

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