

COMMUNICATION

Invisible invaders: A new warning flag for molecularly detected alien species (MODAS) in databases and information systems

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Abstract

Invasive alien species (IAS) threaten biodiversity, ecosystem functioning, and services. Early detection and monitoring are essential for preventing IAS spread and mitigating their impacts. Conventional monitoring approaches rely on physical evidence and frequently fail to detect species in inaccessible habitats, cryptic life stages, or poorly studied taxa. Advances in environmental DNA (eDNA) and other high-throughput molecular tools offer a sensitive alternative that can identify species undetectable by traditional methods. However, current regulatory frameworks require morphological verification, excluding molecularly detected IAS. Here, we propose adding molecularly detected alien species (MODAS) as a warning flag for alien species identified exclusively through molecular data. We define metadata requirements for the MODAS designation, including information on sampling, molecular analyses, DNA marker(s), sequence quality, taxonomic assignment confidence, and reference database. Incorporating MODAS flag into existing IAS databases and information systems would help prioritize validation efforts, support rapid response actions, and improve surveillance coverage, particularly in understudied or vulnerable environments. Moreover, tracking MODAS over spatial and temporal scales can provide critical feedback for refining and advancing IAS monitoring and surveillance approaches. This framework provides a precautionary, complementary approach for integrating molecular biodiversity tools into policy and management, promoting international coordination, and addressing gaps in current IAS monitoring strategies.

KEYWORDS

DNA metabarcoding, early warning systems, environmental DNA, invasive alien species

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INTRODUCTION

Early detection and monitoring of alien species are essential for assessing and managing biodiversity threats and ecological change (Comtet et al., 2015; Pyšek et al., 2020; Simberloff et al., 2005). Consequently, many countries have implemented regulations that incorporate early detection and warning systems to reduce the environmental risks associated with the introduction of alien species. In 2014, the European Union adopted Regulation 1143/2014 on invasive alien species (IAS) to prevent and mitigate their adverse impacts on biodiversity (Cardoso et al., 2021). Similarly, in 2016, the United States issued Executive Order 13751 to prevent the introduction and spread of IAS and support efforts for their eradication or control (Burgos-Rodríguez & Burgiel, 2020; Reaser, 2020). Following these regulations, early warning systems have been developed for the rapid detection of IAS at all life stages and across diverse habitats, even at very low abundances within newly invaded regions (Mehta et al., 2007; Rainford et al., 2020; Simpson et al., 2009). Such early detection is critical, as eradication becomes increasingly difficult and exponentially more expensive once an introduced species manages to reproduce and spread (Epanchin-Niell, 2017; Katsanevakis et al., 2023; Mehta et al., 2007).

Traditionally, IAS records rely exclusively on physical observations and the collection of specimens, either targeted or incidental (Reaser et al., 2020). In some cases, molecular data are extracted from these physical samples and analyzed using DNA barcoding, that is, comparing standardized gene regions (e.g., COI, 18S rRNA) against curated genetic repositories such as NCBI GenBank (<https://www.ncbi.nlm.nih.gov/>) or the Barcode of Life Data System (BOLD, <https://boldsystems.org/>). This process helps verify morphological identifications, resolve taxonomic ambiguities, detect cryptic species (Renner et al., 2024), and estimate the origin region of introduction (Rothman et al., 2016). With the advent of citizen science platforms, images and video footage have also been used as legitimate biodiversity evidence (Johnson et al., 2020). While the prerequisite of physical observation facilitates verifiability, it also introduces limitations and biases. Detection is often constrained in remote areas with lower accessibility or poor visibility (e.g., deep-sea, offshore, sediment-rich environments, subterranean ecosystems), especially for microscopic or cryptic life stages. As a result, IAS databases tend to overrepresent large, well-studied taxa, such as vertebrates, vascular plants, and commercially exploited invertebrates, whereas microorganisms, meiofauna, and many planktonic groups remain underreported (McGeoch et al., 2010). Marine or inland waters in developing countries are less represented than

terrestrial ecosystems, especially in tropical and subtropical regions (Pyšek et al., 2008). These biases reflect gaps in monitoring effort and taxonomic expertise, particularly in regions with limited capacity for species identification or access to trained specialists (McGeoch et al., 2012). Thus, we propose incorporating a new warning flag in IAS databases and information systems to report and document alien species identified exclusively through molecular techniques, namely, MODAS—MOlecularly Detected Alien Species.

While MODAS is proposed as a warning flag, its primary contribution lies in facilitating the integration of molecular detections into decision-making workflows. Recent studies have highlighted that molecular detections often remain underutilized due to the lack of structured pathways linking detection to validation and management action (Fernández Winzer et al., 2025). Within this context, MODAS provides a standardized and interoperable mechanism to identify and track such detections, enabling their incorporation into emerging biosecurity workflows and bridging the gap between detection and response.

MOLECULARLY DETECTED ALIEN SPECIES

Environmental DNA (eDNA) metabarcoding and other high-throughput genomic techniques have revolutionized biodiversity assessments, providing highly sensitive, non-invasive tools for detecting organism presence in an ecosystem (Comtet et al., 2015; Darling & Mahon, 2011; Granqvist et al., 2025). These methods can detect a broad range of organisms, in some cases enabling species detection across all life stages, including those that are cryptic (Hending, 2025), elusive (Beng & Corlett, 2020), rare (Duarte et al., 2023), or merely undetectable by traditional means. Molecular methods can outperform traditional ones as early detection systems; for example, the invasive spotted lanternfly *Lycorma delicatula* was detected using eDNA in 84% of tested North American vineyards, with higher detection confidence, compared with only 36% by visual census (Allen et al., 2021). Following the indication for the presence of the invasive copepod *Pseudodiaptomus marinus* in coastal Mediterranean waters during specific months using metabarcoding, a positive validation was made using physical samples, where it was found in low concentrations (Guy-Haim et al., 2022). Recent efforts to combine molecular methods with citizen science initiatives for large-scale eDNA sampling can further enhance the capacity of these methods for early detection (Katsanevakis et al., 2024). However, as environmental genomic data accumulate globally, a novel

challenge emerges: a rising number of detections of alien species inferred solely from molecular evidence, with no corresponding physical specimens or direct observation (Figure 1).

High-throughput, molecular-based detections can reveal the presence of IAS through molecular traces left in the environment, sometimes years before physical evidence is obtained via traditional monitoring methods. This lag between molecular detection and morphological confirmation illustrates the value of integrating these molecular tools in early warning systems. For instance, larvae of the Red Sea gastropod *Nerita sanguinolenta* were detected in an eastern Mediterranean anticyclonic eddy by metabarcoding of plankton samples in 2018, 1 year before the first adult individual was observed on shore (Belkin et al., 2022; Rabi et al., 2020). The invasion fronts of two species of Asian carps in Illinois, USA, were delimited using eDNA, 8 months earlier than the detection of these species using traditional fisheries surveillance tools (Jerde et al., 2011). A parasitic anemone was detected using plankton metabarcoding, years before obtaining physical evidence (Iakovleva et al., 2024).

Species detected solely through molecular methods without physical observation may be newly introduced, transient, or even established, but remain formally “invisible” under current regulatory frameworks. We propose assigning a MODAS flag to denote species identified as non-native in a given region based exclusively on molecular data, typically from eDNA metabarcoding or metagenomic surveys of soil, water, plankton, or other

multispecies communities. The MODAS flag would serve as a critical early warning signal, helping scientists and environmental managers prioritize and refine validation efforts and improve rapid response measures. For example, if a rock-dwelling subtidal bivalve is flagged as a MODAS, validation efforts could focus on shallow rocky habitats.

Although molecular-based species detection methods have transformed biodiversity assessments, their integration into IAS monitoring programs remains limited (Beng & Corlett, 2020). While DNA barcoding is widely accepted for species identification (Antil et al., 2023), metabarcoding and metagenomics are still viewed with skepticism in regulatory contexts, despite increasing reliability and standardization. This is partly due to concerns over false positives (Darling et al., 2021), incomplete or erroneous reference databases (Harris, 2003; Locatelli et al., 2020; Pentinsaari et al., 2020), and contamination (Sepulveda et al., 2020). Short universal amplicons used in eDNA metabarcoding (e.g., 18S rRNA v9 ~150 bp (Amaral-Zettler et al., 2009), and COI mtDNA marker for freshwater macroinvertebrates ~180 bp (Vamos et al., 2017)) often lack the taxonomic resolution required for species-level taxonomic assignments. However, new tools are rapidly developing to address these challenges, including quality-controlled taxonomically verified molecular reference databases (Leray et al., 2022; O’Brien et al., 2024), confidence scoring (Polanco et al., 2025), probabilistic taxonomic assignment (Gold, Curd, et al., 2021), protocols to mitigate other sources of errors

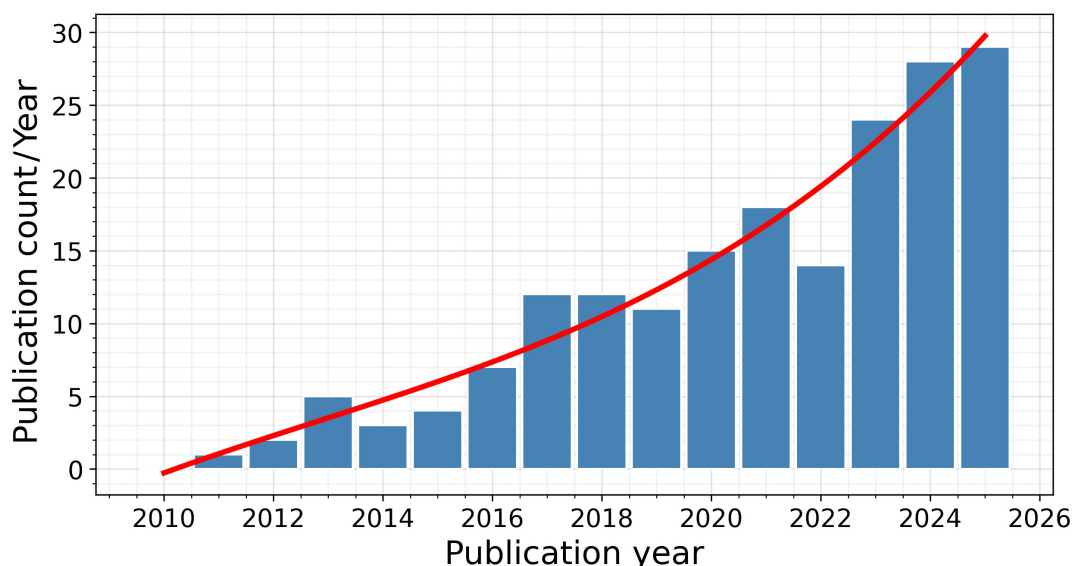


FIGURE 1 The publication trend of scientific articles on molecularly detected alien species (MODAS) estimated from ISI Web of Science search using the search string (“alien species” OR “invasive species” OR “introduced species” OR “non-indigenous species” OR “non-native species” OR neobiota) AND (eDNA OR “environmental DNA” OR meta?barcoding OR meta?genomics OR high?throughput) AND (“first record” OR “new record” OR “early detection”). The search was run on 7 April 2026.

(Burian et al., 2021), and newly developed primers designed to identify targeted taxonomic groups to the species level (e.g., 12S mtDNA MiFish primers used for the detection of marine and freshwater fish; Miya et al., 2015; Stern et al., 2025, and 16S mtDNA MiDeca used for the detection of decapod crustaceans; Komai et al., 2019). Consequently, the quality and reliability of eDNA metabarcoding data are steadily improving.

IAS OCCURRENCE CATEGORIES IN DATABASES AND INFORMATION SYSTEMS

Biodiversity and IAS species databases support decision-making by integrating geospatial tools and region-specific data. The classification of alien species in these databases has progressively evolved to facilitate consistent monitoring, risk assessment, and evidence-based policy-making (Turbelin et al., 2017). A core component of this effort is the use of standardized occurrence categories, which define the nature and degree of presence of a species in a given region. Major frameworks, such as the European Alien Species Information Network (EASIN) (Katsanevakis et al., 2012), the Global Register of Introduced and Invasive Species (GRIIS) (Pagad et al., 2018), the Global Invasive Species Database (GISD) (Ricciardi et al., 2000), the Invasive Species Compendium (CABI-ISC) (Diaz-Soltero, 2022), the Pacific Invasive Species Database (PISD) (Fornwall & Loope, 2004), and the World Register of Introduced Marine Species (WRiMS) (Costello et al., 2021), apply these categories to distinguish between stages of establishment and levels of certainty.

These occurrence categories typically include: (1) Casual/transient: species detected sporadically, often due to recent or repeated introductions, but not known to form self-sustaining populations; (2) Established/naturalized: species with stable, self-maintaining populations in the wild, no longer reliant on human-mediated introduction; (3) Eradicated/extirpated: species that were previously present but have been removed or become extinct in a specific region; (4) Absent: species not currently found in a region despite previous records; (5) Uncertain/questionable: species with ambiguous or unresolved presence due to insufficient evidence or taxonomic issues; and (6) Historic/formerly present: species with a known past presence but currently not detected (Groom et al., 2019; Haubrock et al., 2024; Soto et al., 2024).

While MODAS records could hypothetically be placed under the “Uncertain/questionable” category, doing so conflates novel molecular detections with taxonomically ambiguous or unreliable records, and fails to inform

scientists and policy-makers about the potential of employing molecular methods to further detect new introductions (Clarke et al., 2021; Kearney, 2002). We therefore propose MODAS as a complementary warning flag within IAS databases, providing a framework to record molecular signals that can be tracked and validated. Rather than replacing existing classification frameworks or taxonomic expertise, this approach strengthens them by providing an additional layer of early warning, while remaining compatible with existing data standards and infrastructures. In practice, a record could retain its existing occurrence category while being flagged as MODAS when the evidence basis is molecular-only, enabling compatibility with emerging Darwin Core vocabularies for alien species and with infrastructures that aim to support standardized metabarcoding publication workflows, such as GBIF’s Metabarcoding Data Toolkit (GBIF, 2026).

Tracking MODAS across spatial and temporal scales could also yield critical feedback for refining monitoring methods and improving IAS surveillance strategies. Furthermore, bordering countries could get an early warning of potentially “doorknocker” species (Ekrem et al., 2023; Stern et al., 2025). This approach aligns with the precautionary principle (Persson, 2016), particularly in secluded, poorly monitored, or sensitive habitats such as deep-sea, offshore, or protected areas (Gold, Sprague, et al., 2021; McClenaghan et al., 2020; Saccò et al., 2025).

REQUIREMENTS FOR REPORTING OF MODAS

The implementation of MODAS is intended to function within broader decision-support frameworks that guide the progression from detection to validation and action. For example, workflow-based approaches have been proposed to systematically evaluate molecular detections by cross-referencing them with species lists, assessing evidence quality, and prioritizing follow-up actions (Fernández Winzer et al., 2025). MODAS can serve as a standardized input into such frameworks, enabling consistent treatment of molecular detections across systems.

A species may be flagged as MODAS if it meets the following conditions: (1) The molecular signal must occur in a geographic region where the species is not considered native, according to established biogeographic records (e.g., GBIF, OBIS, WoRMS) or authoritative databases. This criterion requires that the alien status of the taxon is reasonably well resolved; cryptogenic species or with uncertain native ranges should not be assigned MODAS until their biogeographic status is clarified, to avoid propagating classification errors. (2) The species

must be detected through molecular techniques, such as DNA barcoding, eDNA metabarcoding, qPCR, or shotgun metagenomics. (3) Lack of morphological confirmation, that is, no concurrent or prior morphological, visual, or specimen-based confirmation of the species' presence exists at the site of detection. Once such evidence becomes available, the relevant occurrence category should be assigned.

Further methodological considerations can enhance the reliability of a MODAS designation. These include repeated detections across independent samples, replicates, sites, or time points, and the use of multiple molecular markers (Guy-Haim et al., 2022; Varrella et al., 2025). Adherence to such standards minimizes false positives due to contamination or PCR/sequencing errors. To address the methodological caveats associated with metabarcoding, and to ensure transparency and reproducibility, each MODAS record must be accompanied by metadata, including: (1) sampling date and location, (2) collection and extraction protocols (e.g., water filtration, sediment extraction, plankton collection), (3) DNA marker(s) used, (4) sequence quality metrics (error rate estimates), (5) taxonomic assignment confidence (e.g., % identity), and (6) reference database details with accession numbers. These metadata requirements are consistent with emerging community standards for minimum information reporting and FAIR eDNA data management, which aim to improve reproducibility, comparability, and interoperability across metabarcoding workflows (Takahashi et al., 2025).

Environmental data (e.g., temperature, salinity, humidity, pH, solar radiation) may also offer insights on the quality of the obtained genetic material (Barnes et al., 2014; McCartin et al., 2022; Valentin et al., 2021). Newly developed tools, such as GBIF Metabarcoding Data Toolkit (GBIF, 2026), can facilitate the identification of MODAS records from metabarcoding and eDNA datasets, which can later be submitted to IAS databases. It is important to note that the MODAS flag is intended to be dynamic and open to revision, with entries updated once new evidence becomes available, including morphological confirmation, additional molecular surveys, or observed range expansions.

MODAS detections do not necessarily indicate established populations. Molecular signals may reflect transient presence, passive transport (e.g., via currents, ballast water, or biological vectors), or legacy DNA (Deiner et al., 2017; Shogren et al., 2017). Accordingly, MODAS should be interpreted as a precautionary signal rather than as a confirmation of establishment. At the same time, the MODAS flag can enable provisional inclusion of a species in alien species databases, surveillance reports, or monitoring programs, thereby supporting

prioritization of targeted verification followed by rapid response strategies such as localized eradication or containment. The MODAS designation is not meant to replace existing categories but to complement and strengthen them by offering a clearly defined “early warning” status. This layered approach allows for a stepwise confidence model, similar to strategies in disease surveillance or biodiversity assessments (e.g., “probable,” “confirmed,” “established”). While a quantitative decision framework is beyond the scope of this communication, implementation of the MODAS flag would be operationalized through the definition of false-positive/false-negative rates, minimum evidence thresholds by marker, taxon or environment, rules for contamination and legacy DNA, and appropriate governance for upgrading or downgrading available records.

Beyond its value as an early warning tool, the adoption of MODAS could guide targeted investment toward training and supporting taxonomists, and direct funding to the generation of curated reference material, fostering synergies between molecular ecology and traditional systematics. Standardizing the MODAS concept across global and regional alien species databases, taxonomic groups, and regions could also encourage international coordination and data sharing among scientists, decision-makers, and other stakeholders.

AUTHOR CONTRIBUTIONS

Tamar Guy-Haim conceptualized the study, performed the analyses, acquired funding, and prepared the original draft of the manuscript. Nir Stern, Elizabeta Briski, and Stelios Katsanevakis contributed to the review and editing of the manuscript. Eleni Christoforou contributed to funding acquisition and participated in the review and editing process. All coauthors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data were retrieved from the ISI Web of Science (<https://www.webofscience.com/>). Query details for retrieving the relevant data are as follows: Topic search (“alien species” OR “invasive species” OR “introduced species” OR “non-indigenous species” OR “non-native species” OR neobiota) AND (eDNA OR “environmental DNA” OR meta?barcoding OR meta?genomics OR high?throughput) AND (“first record” OR “new record” OR “early detection”). The initial search was run on 15 April 2025 and an updated search was performed on 7 April 2026.

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