

Collective and harmonised high throughput barcoding of insular arthropod biodiversity: toward a Genomic Observatories Network for islands

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Abstract

Our current understanding of ecological and evolutionary processes underlying island biodiversity is heavily shaped by empirical data from plants and birds, although arthropods comprise the overwhelming majority of known animal species. This is due to inherent problems with obtaining high-quality arthropod data. Novel high throughput sequencing approaches are now emerging as powerful tools to overcome such limitations, and thus comprehensively address existing shortfalls in arthropod biodiversity data. Here we explore how, as a community, we might most effectively exploit these tools for comprehensive and comparable inventory and monitoring of insular arthropod biodiversity. We first review the strengths, limitations and potential synergies among existing approaches of high throughput barcode sequencing. We consider how this can be complemented with deep learning approaches applied to image analysis to study arthropod biodiversity. We then explore how these approaches can be implemented within the framework of an island Genomic Observatories Network (iGON) for the advancement of fundamental and applied understanding of island biodiversity. To this end, we identify seven island biology themes at the interface of ecology, evolution and conservation biology, within which collective and harmonised efforts in HTS arthropod inventory could yield significant advances in island biodiversity research.

Introduction

Islands have long been a focus for evolutionary and ecological understanding (Warren et al., 2015), largely due to their limited geographic extent, long-term isolation, replicated nature, simplified biota relative to continental settings, high levels of endemism, and diverse ecological settings. At the same time, their flora and fauna are increasingly at risk from global change relative to continental settings for some of the very same reasons that have attracted scientific interest. Depauperate communities that have evolved in isolation may be more susceptible to invasive species (Bellard, Rysman, Leroy, Claud, & Mace, 2017; Spatz et al., 2017; Borges et al. 2020). Within the context of ongoing climate change, island biodiversity requires specific attention because of its increased vulnerability for multiple reasons (see e.g. Manes et al., 2021; Veron, Mouchet, Govaerts, Haevermans, & Pellens, 2019). When climate change is combined with other impacts of increasing human population size and economic development, such as habitat modification and degradation, the challenge for managing and conserving insular biodiversity presents itself as being immediate and large-scale (Russell & Kueffer, 2019). It is not only the loss of species and their interactions that is particularly relevant on islands, but also the loss of unique evolutionary history (phylogenetic and functional diversity), reflecting the loss of unique adaptations to the environment (Sayol et al., 2021; Soares, de Lima, Palmeirim, Cardoso, & Rodrigues, 2021).

Given the above, there are pure and applied scientific reasons for scaling up our understanding of island biodiversity. Scientifically, there is still much to be gained from the investigation of insular biotas (Warren et al., 2015; Patiño et al., 2017; Whittaker, Fernández-Palacios, Matthews, Borregaard, & Triantis, 2017), but with many questions remaining open due to limited arthropod data (Table 1). Our current understanding of ecological and evolutionary processes within islands, and most of the proposed island biodiversity patterns, rules and models, largely derive from empirical data on plants and birds (e.g. Matthews, Rigal, Triantis, & Whittaker, 2019; Valente et al., 2020; Veron, Haevermans, Govaerts, Mouchet, & Pellens, 2019). While invertebrates played a key role in the early developments of island biology (MacArthur & Wilson, 1963; MacArthur & Wilson, 1967; E. Wilson, 1961; E. O. Wilson, 1959), and typically represent the vast majority of insular biodiversity, invertebrate data pertaining to range size and co-occurrence remain under-represented in existing datasets. This can be explained, in part, by the relative difficulty of obtaining such data. It is recognised that invertebrates play a fundamental role in ecosystem processes and services (Dangles & Casas, 2019), and the potential negative impacts of introduced invertebrate species are also well appreciated, with an estimated annual cost of more than 20k million US\$ (Diagne et al., 2021). However, understanding of the contribution of invertebrate species to ecosystem resilience, and their vulnerabilities, remain strongly data-limited (Cardoso, Erwin, Borges, & New, 2011; Cardoso & Leather, 2019; Harvey et al., 2020).

A recent initiative calling for the integration of arthropods within the monitoring of insular forest biodiversity also highlights the potentially prohibitive workload for this, even for a limited subset of arthropod biodiversity (Borges et al., 2018). The broad characterisation of invertebrate communities is a universal challenge, largely caused by logistical constraints associated with both the sorting of large volumes of invertebrate material, and its classification to species. However, addressing these challenges through the application of genome-based sampling and taxonomic assignment is an area of intense activity (e.g. Arribas et al., 2021; Kennedy et al., 2020; Piper et al., 2019), currently only paralleled by recent advances in the application of machine learning for the identification of taxa from image processing (Wührl et al., 2021; Årje et al., 2020; Valan, Makonyi, Maki, Vondráček, & Ronquist, 2019). Both DNA-based and image-based automated identification have the potential to exponentially accelerate arthropod diversity quantification in the near future (Høye et al., 2021; Wührl, et al., 2021). It is now timely to consider how these developments might be integrated to advance the understanding, management and conservation of insular biotas (Fig. 1, Table 1). Here, we focus on the arthropod fraction of terrestrial invertebrate biodiversity to provide a collective opinion of how, as a community, we might most effectively exploit new technologies and techniques to inventory and monitor insular arthropod biodiversity.

High throughput barcode sequencing of arthropods at scale

Open questions in island biology arArthropods offer high potential for structured sampling to obtain data-

rich site-based community data, through the use of multiple complementary sampling methods (Montgomery, Belitz, Guralnick, & Tingley, 2021). For nearly all sampling methods, sorting of samples and classification to species are likely to be substantial challenges, and thus a limitation to sampling at scale. For many groups, regional taxonomic knowledge is incomplete, and for those groups that benefit from a robust taxonomic framework, sorting and species assignment may still be complicated by a lack of specific taxonomic expertise. Molecular barcode-based sequencing tools have already helped to overcome the challenges of taxonomic assignment and facilitate new species discovery and monitoring (e.g. deWaard et al., 2019; Hebert, Cywinska, Ball, & DeWaard, 2003; Ronquist et al., 2020), and there are broader benefits for the characterisation of diverse arthropod communities using high throughput sequencing (HTS) barcoding (Hajibabaei, Baird, Fahner, Beiko, & Golding, 2016). HTS can be employed at the scale of individual specimens or bulk community samples (see Kennedy et al. (2020) and sections below), and both can greatly reduce existing limitations for identifying and understanding biodiversity patterns and processes across entire arthropod assemblages on islands. Additionally, even in the absence of a local reference library, when HTS barcoding is coupled with the use of the universal mitochondrial DNA (mtDNA) barcode region (Andújar, Arribas, Yu, Vogler, & Emerson, 2018), taxonomic assignment is aided by the more than eight million barcode sequences within the BOLD repository.

Multiplex barcoding and whole organism community metabarcoding

While classical barcode sequencing involves the individualisation of both the PCR and sequencing reactions, HTS platforms now offer the opportunity to pool thousands of amplicons from individual specimens via tagged amplicon sequencing (Creedy et al., 2020; Hebert et al., 2018; Shokralla et al., 2014; Srivathsan et al., 2019, 2021; de Kerdrel et al. 2020). This can be scaled up to 10,000 multiplexed individuals within a single MinION flow cell (Srivathsan et al., 2019, 2021) or several hundred thousand for one lane of NovaSeq 6000 when a reduced length “mini barcode” is used (Yeo, Srivathsan & Meier, 2020). HTS multiplex barcoding provides a direct link between DNA sequences and the individuals from which they were amplified. This has several advantages. It allows one to sort physical specimens to putative species and to resolve taxonomic disagreements between barcodes and other data (Wang, Srivathsan, Foo, Yamane, & Meier, 2018). This is necessary when the associated sequence appears unusual (e.g. unexpectedly high sequence divergence) or species delimitation approaches with different algorithms return conflicting results (Meier et al., 2021). It also allows one to return to the DNA extract, should there be interest in further exploring the nuclear genome, diet content or microbiome of specific specimens (Kennedy et al., 2020). Another very obvious advantage is that abundance estimates can be directly extracted from the DNA sequence data.

In contrast to multiplex barcoding, whole organism community DNA (wocDNA) metabarcoding (Andújar et al., 2018; Creedy et al., 2021; Yu et al., 2012) involves a single DNA extraction for multiple individuals from multiple species, that is subsequently PCR amplified and sequenced, typically using the Illumina platform. This reduces the individualised processing of specimens, which is particularly relevant for hyperdiverse (and minute specimen) arthropod assemblages (e.g. Arribas et al., 2016; Creedy et al., 2019) and/or high numbers of community samples (e.g. for long-term or broad-scale approaches). However, there are a number of ways in which the information content of wocDNA metabarcode data is different from multiplex barcode data, either requiring additional data processing or placing limits on inferences that can be derived. An important feature of wocDNA metabarcode sequence output is the difficulty to discern spurious sequences (PCR and DNA sequencing artefacts, contamination, nuclear copies, or different combinations of these) from real (but low abundance) sequences in the community sample. With appropriate laboratory protocols, design and bioinformatic processing, contamination issues and PCR and DNA sequencing artefacts can be substantially reduced (e.g. Alberdi, Aizpurua, Gilbert, & Bohmann, 2018; Creedy et al., 2021). It has also recently become possible to effectively remove nuclear copies of mtDNA sequences, providing for haplotype-level resolution from wocDNA metabarcode data (Andújar et al., 2021). Within wocDNA metabarcode data, there is no correspondence between sequences and the individual from which they are derived. While biodiversity patterns can still be explored without taxonomic assignment, species-level taxonomic assignment is generally a desirable feature, and in this case, can be only achieved with taxonomically assigned barcode reference sequences. Even without species-specific reference libraries, arthropod sequence assignment to some taxo-

nomic level can be achieved using public repositories (e.g. GenBank or BOLD). Finally, the extrapolation of abundance data from metabarcode sequence output is complicated, but several promising approaches for deriving abundance data from standardised samples have been developed (e.g. Ji et al., 2020; Krehenwinkel et al., 2017; Luo, Ji, & Yu, 2022).

The choice of HTS barcoding approach to catalogue arthropod biodiversity will be dependent on the specific objectives to be addressed. However, there are potential synergies from combining high throughput generation of community-level data by wocDNA metabarcoding, together with vouchered sequencing by multiplex barcoding. Vouchering may be considered unnecessary when well-parameterized reference libraries are available, but it is otherwise an essential consideration for future taxonomic assignment of metabarcoding reads and for completing reference barcode databases. Individualised and validated barcodes generated by multiplex barcoding are also of particular relevance for the bioinformatic processing of metabarcode reads (Andújar et al., 2021).

Robotics, artificial intelligence and the integration of image analysis and barcode sequencing

Exciting advances are being made in the areas of robotics and image analysis for arthropods. Species identification via image analysis is currently based on convolutional neural networks (CNNs), a specific tool in the field of deep learning (DL), where complex image patterns are classified taking advantage of training sets (Valan et al., 2019, Valan, Vondráček, & Ronquist, 2021). However, the training of CNNs requires large sets of training images whereby each image has to be labelled with reliable taxonomic information. Perhaps not surprisingly, such datasets are available for bees and butterflies (Buschbacher, Ahrens, Espeland, & Steinhage, 2020), but are largely missing for the bulk of arthropods collected by standardised trapping. The challenge is to generate these sets, and this is where a combination of robotic specimen handling and HTS barcoding can help. Robotics can generate the images and HTS barcoding can sort images to putative species. Recently, Wühl et al. (2021) presented a first-generation robot for this purpose. It detects, images and measures specimens, before moving them into a 96-well microplate for DNA barcode sequencing. This approach opens the door for a transition away from multiplex barcode sequencing of all individuals toward taxonomic assignment by image recognition, as images with barcode sequences contribute to training images for machine learning. However, it remains unclear whether automated image-based approaches alone will eventually reach the approximately species-level resolution obtained with sequence data. Image-based specimen identification could, nevertheless, be used as an external validation of molecular-based diversity estimations at, for example, genus level. Similarly, image analyses can yield information on sample biomass and abundance (Ärje et al., 2020; Schneider et al., 2022, Wühl, 2021).

Genomic Observatories: a framework for harmonised high throughput barcode sequencing of island arthropods

The biodiversity, ecology, and evolution of island arthropod communities can be studied at unprecedented scales and resolution through the individual or joint application of (i) wocDNA metabarcoding, (ii) barcode reference libraries, (iii) multiplex barcoding and (iv) image analyses. Harmonisation across the first three approaches can also provide for a common data currency, facilitating comparisons and synthetic analyses across independent studies. By incorporating the universally accepted arthropod barcode region of the mitochondrial cytochrome oxidase subunit I (COI) gene into wocDNA metabarcoding (Andújar et al., 2018; Elbrecht et al., 2019), the COI barcode region can act as a directly comparable species tag across any given study, transcending potential taxonomic assignment errors within individual studies. The Genomic Observatory concept, within which HTS serves as a core tool for biodiversity assessment (Arribas et al., 2021a), provides a solid foundation for implementing genome-based inventory and monitoring of insular arthropod biodiversity.

Harmonised HTS data generation and bioinformatic workflows for general arthropod inventory and assessment are emerging (e.g. Arribas et al., 2022; Creedy et al., 2021; Srivathsan et al., 2021). However, more development is needed for an inclusive range of sampling protocols that can capture important arthropod fractions of biodiversity on islands (see Montgomery et al., 2021 for a review). For terrestrial fractions of

arthropod biodiversity, these can be developed as submodules within the recently proposed framework of Arribas et al. (2022), taking advantage of their proposed downstream submodules for the processing and sequencing of samples.

In addition to the need for harmonised data generation protocols, there are other generic obstacles for Genomic Observatories that need to be addressed for an efficient island Genomic Observatories Network. One important challenge is to ensure that metabarcode data conform to Findable, Accessible, Interoperable and Reusable (FAIR) Data Principles (Wilkinson et al., 2016), such that new wocDNA metabarcode and multiplex barcoding data sets can be cross-referenced to previous work. In the same way that cross-referencing sequence reads to barcode sequence repositories can assign taxonomy and clarify species origins, additional cross-referencing to a metabarcode sequence repository would facilitate understanding the structure of community similarity over a range of spatial scales. The GEOME (Genomic Observatories Metadatabase; Deck et al., 2017; Riginos et al., 2020) initiative offers a very useful platform, facilitating FAIR data archival practices. GEOME also facilitates DNA data sharing through the deposition of raw genetic data to the Sequence Read Archive (SRA, www.ncbi.nlm.nih.gov/sra), while maintaining persistent links to standard-compliant metadata held in the GEOME database. Achieving seamless cross-referencing among *de novo* wocDNA metabarcode sequences, multiplex barcoding sequences and repositories of both barcode sequences and wocDNA metabarcode sequences has the potential to dramatically extend the scope and reach of such data.

Island Genomic Observatories - a network for island arthropod conservation and broader biodiversity understanding

With strategic implementation, an island Genomic Observatories Network (iGON) has the potential to advance understanding in three areas, the first and more fundamental of which is that of knowledge acquisition and transfer for conservation and sustainable management. Arthropods dominate the native and endemic fractions of island biodiversity, while also representing substantial invasive species risk, but are data deficient compared to vertebrates and plants. HTS barcoding can greatly contribute to knowledge deficits concerning species inventory, species distributions, the geographic structuring of genetic variation within species, and the factors that explain this structure (e.g. Arjona et al., 2022 this issue). With specific regard to islands, the generation of HTS barcode data within an iGON opens the door to improved investigation of fundamental island biogeographic theory using arthropods (e.g. Andújar et al., 2022; Armstrong et al., 2022; Lim et al., 2021 this issue). Finally, island communities can be leveraged to address the recognised need for integration across the disciplines of macroecology and macroevolution, where synthesising data, tools and perspectives is required (McGill et al., 2019). The often simplified and replicated nature of island ecosystems, together with increasingly available technology to characterise the arthropod fractions of their biodiversity, provides a profitable arena for such integration.

To explore the potential of an iGON for the advancement of fundamental and applied biodiversity understanding, we use two recent reviews as a guiding framework, focussed on open questions in island biology (Patiño et al., 2017) and the unification of macroecology and macroevolution (McGill et al., 2019). Within this framework, we identify key themes within which collective and harmonised efforts in HTS arthropod inventory (either in isolation or in concert with other approaches) could yield significant advances in island biodiversity research (see Fig. 1, Table 1). Some of these themes include questions related to basic properties of species diversity of island arthropods, compared to more studied vertebrate groups, while others pertain to fundamental research areas that have been constrained by access to data.

1. Global patterns and drivers of island arthropod biodiversity

Studies of global-scale patterns and drivers of island biodiversity rely on the existence of island species inventories. Arthropod species lists for islands have been leveraged for comparative analyses to infer the processes that shape variation in species richness (e.g. Chown, Gremmen, & Gaston, 1998; Santos, Fontaine, Quicke, Borges, & Hortal, 2011; Triantis, Economo, Guilhaumon, & Ricklefs, 2015). However, it is increasingly recognised that many arthropod species are still not formally described (commonly described as the Linnean

shortfall, Lomolino, 2004), strongly hampering inferences of richness, diversity and endemism (e.g. Cicconardi, Fanciulli, & Emerson, 2013; Emerson, Cicconardi, Fanciulli, & Shaw, 2011; Legros, Rochat, Reynaud, & Strasberg, 2020), and hence, conservation efforts (Cardoso et al., 2011). A structured HTS inventory of arthropod communities across islands holds great potential to overcome this shortfall by providing a way forward to overcome traditional challenges of identification. HTS barcoding allows for communities to be inventoried independently of the degree of undescribed species present, while simultaneously highlighting species that are either described and absent from a local reference library, or undescribed. This opens the door for inclusive inventories that extend to the typically small and cryptic taxa fractions of arthropod diversity that are associated with high levels of undescribed species, the so called “dark taxa” (Hartop, Srivathsan, Ronquist, & Meier, 2021; Hausmann et al., 2020). As proof of concept, Yeo et al. (2021) implemented multiplex barcoding to inventory the tropical arthropod fauna of Singapore across six different forests and a freshwater swamp. They generated 140,000 barcoded specimens belonging to ca. 8,500 species and identified an overlooked hotspot of insect diversity within the mangrove habitat. Similarly, Arjona et al. (2022, this issue) applied wocDNA metabarcoding to characterise the coleopteran fauna sampled from soils within an island cloud forest, highlighting the power of HTS for both detecting unrecorded species and species discovery. They additionally demonstrated the value of barcode reference libraries, even if incomplete, for efficient bioinformatic processing to achieve reliable haplotype data (Andújar et al., 2021), and in doing so, provide strong evidence for taxonomic inflation in the absence of such processing. Complete island arthropod inventories (i.e. from exhaustive sampling and encompassing undescribed species) that are comparable across different insular systems (by direct comparison of DNA barcodes) can be used to address fundamental topics within island biology, for which understanding is still limited. These include: (i) the relationships among island area, geological age, elevation and isolation and arthropod species richness; (ii) the biogeographic processes driving island species-area relationships (SARs), and (iii) how arthropod SARs vary among different arthropod fractions and among different island contexts, including oceanic, continental-shelf, continental fragment and habitat islands.

WocDNA metabarcoding can be applied to generate vast amounts of site-based data, and if combined with multiplex barcoding, barcoded vouchers can be retained for specific taxonomic reference. Such HTS barcoding data opens the door for the phylogenetic placement of potentially all sampled species when combined with appropriate backbone trees (e.g. Balaban, Sarmashghi, & Mirarab, 2018). With the development of mitochondrial metagenomics (Crampton-Platt, Yu, Zhou, & Vogler, 2016), backbone trees can now be generated with good resolution for major arthropod lineages (e.g. Arribas et al., 2020; Tang et al., 2019). Thus, by returning to multiplex barcode DNA extracts or strategically sampling vouchers from wocDNA samples prior to bulk DNA extraction, such backbone trees for an island fauna can be obtained. Assemblage level phylogenetic trees then provide the needed framework to ask, for example, how taxonomic and phylogenetic diversity vary across gradients of interest, within islands, among islands, and between islands and ecologically comparable continental areas.

2. Island community assembly of arthropod biodiversity

Understanding how biological communities form, and why they differ spatially and temporally, is a key objective in ecology. The integration of phylogeny into the analysis of community ecology has provided new dimensions for comparing and contrasting communities, within which insular environments have proven to be useful sampling frameworks (Emerson & Gillespie, 2008; Shaw & Gillespie, 2016). Island systems can yield significant arthropod structuring and turnover over relatively limited spatial scales and across entire assemblages (e.g. Salces-Castellano et al., 2021). Such limited spatial dimensions allow for fine-scale but geographically representative community-level sampling to characterise the community assembly process. When executed across multiple islands and coupled with remote sensing data, there is much potential for an improved understanding of the factors that shape such patterns (Bush et al., 2017).

Community-scale investigation to describe patterns and infer processes for island biodiversity requires multiple site-based characterisations of communities, which is a clear bottleneck in the case of the arthropod biodiversity. Existing studies in this area have been limited to specific arthropod lineages, such as beetles

or spiders, for which conventional taxonomical and molecular processing is time-consuming (e.g. Malumbres-Olarte et al., 2021; Antonia Salces-Castellano et al., 2020). HTS barcoding provides an opportunity to increase both the taxonomic and geographic scale of arthropod community sampling. When combined with distribution data across gradients (e.g. elevation, disturbance, island age) or trait data (e.g. dispersal ability, body size), the opportunities for macroevolutionary and macroecological unification become tangible. As a proof of concept, Lim et al. (2021) applied wocDNA metabarcoding to characterise complete arthropod communities across elevational gradients within the island of Hawaii. They revealed that climatic niche conservatism is an important factor shaping ecological assembly across elevation, thus implicating topographic complexity as an important driver of diversification. Similarly, wocDNA metabarcoding approaches to characterise soil arthropod assemblages on the islands of Tenerife and Cyprus have revealed strong habitat filtering and dispersal limitations as drivers of community assembly within islands (Andujar et al., 2022; Nogueras et al., 2022 this issue).

Islands, particularly remote islands, offer much potential for integrating intraspecific-scale analyses together with phylogenetic sampling for the investigation of community assembly. Speciation represents an important contribution to both the origin and evolution of community structure on remote islands (Shaw & Gillespie, 2016), thus providing opportunities to link diversification patterns within species to patterns of speciation at higher levels. Community-level intraspecific sampling on islands has seen less implementation, and it is here that HTS barcoding can play an important role. As well as recording species presence, HTS barcoding provides a measure of haplotype variation within and across communities, thus addressing the traditional Darwinian shortfall (defined as the lack of knowledge regarding the evolution of lineages, species, and traits; Diniz-Filho, Loyola, Raia, Mooers, & Bini, 2013) for arthropod island faunas. Thus, alpha and beta diversity can be analysed for hyperdiverse arthropod communities from genetic to different levels of taxonomic hierarchy to understand how community-level processes drive macroecological and macroevolutionary patterns. Processes that can be characterised include the relative importance of stochasticity, isolation by distance, and habitat or host-associated differentiation (e.g., see Andujar et al., 2022; Nogueras et al., 2022; Arribas, Andujar, Salces-Castellano, Emerson, & Vogler, 2021). Additionally, the extent to which species diversity and genetic diversity covary can be derived from such data (Vellend et al., 2014; Vellend, 2010). Overcast, Emerson, & Hickerson, (2019) have recently described a mechanistic model of community assembly that can generate linked patterns of abundance and genetic diversity under an assumption of joint ecological and evolutionary neutrality, allowing for the estimation of community abundance structure using only intraspecific genetic variation. As proof of concept, this study demonstrated that the abundance structure of spiders on the island of Reunion could be accurately estimated from intraspecific variation from barcode data (Emerson et al., 2017). Further theoretical developments will be needed to fully exploit the potential of genetic community-level data for unifying macroevolution and microevolution, together with macroecology and microecology, and thus further advance island biogeography theory (see Overcast et al., 2022, for a review).

3. Drivers of island arthropod speciation

Traditional approaches to speciation research typically analyse a limited number of species with great detail, necessitating accurate estimation of population and phylogenetic histories, and the limitations of single-locus markers for such purposes are well recognized (e.g. Bazin, Glemin, & Galtier, 2006; Toussaint et al., 2015). However it has been shown that, across multi-species comparisons, potentially idiosyncratic single marker signals within a subset of arthropod species may be outweighed by common community level signatures (e.g. Salces-Castellano et al., 2020; Scalercio et al., 2020). As the histories of individual species complexes may themselves be idiosyncratic, having noisy data across hundreds or even thousands of species may, in some cases, be more revealing of general patterns and processes in a region than deeper sequencing of a more limited number. With the ability to now obtain reliable haplotype-level data from metabarcode sequence output (Andujar et al., 2021), it is possible to implement both multiplex barcode and wocDNA metabarcoding to explore both the patterns and drivers of diversification and speciation across arthropod assemblages. Andujar et al. (2022, this issue) demonstrate the implementation of such a metaphylogeographic approach with wocDNA barcoding to understand the relative roles of ecological and geographical drivers for diversification

among soil arthropods within a single oceanic island. Extending such an approach across multiple islands within archipelagos can provide baseline data on the relative importance of ecological and geographical speciation within many as yet largely unstudied fractions of arthropod diversity. When contrasted with existing data for plants, vertebrates, and more easily studied invertebrate groups, a fuller understanding of: (i) dispersal dynamics within and among islands; (ii) the role of environment in structuring genetic variation within species, and; (iii) their implications for speciation, will emerge. Recent work also demonstrates how a single locus community-level approach can inform about the relative importance of specific traits for diversification within islands (Salces-Castellano et al., 2021). In conjunction with barcode reference libraries with trait data (e.g. body size, dispersal ability, niche) multiplex barcoding and wocDNA metabarcoding can be used to scale up both geographic and taxonomic sampling to identify functional traits associated with arthropod diversification within and across island systems.

4. Arthropod species abundances, endemism and rarity within islands

The Prestonian shortfall is defined as the lack of knowledge about the abundance of species and their population dynamics in space and time (Cardoso et al., 2011). This shortfall is extremely pronounced in the case of arthropods (Cardoso & Leather, 2019), likely associated with the high diversity and complexity of both individual population trends and species interactions of local arthropod communities. HTS barcoding, particularly multiplex barcoding, has much potential to address this shortfall. Generating abundance estimates through HTS barcoding for insular arthropod communities is a potentially rich source of information for empirical testing of island biogeographic theory. This is particularly relevant for questions regarding arthropod SADs within islands (e.g. Borda-De-Agua et al., 2017), and questions at the intersection of species abundances patterns and the processes of speciation and extinction. Such abundance data can now be directly generated for complete arthropod assemblages, even in the absence of formal species description, using multiplex barcoding (e.g. see Srivathsan et al., 2021). PCR free metagenomic approaches can also provide abundance estimates (Ji et al. 2020). In addition to this, the integration of image analysis, together with either (i) multiplex barcoding, or (ii) wocDNA metabarcoding, can further remove limitations of scale. Beyond specific interest in island biogeographic process, it has also been pointed out that sampling across islands can provide for a more general understanding of how and why species abundances change through community assembly (Warren et al., 2015). The neutral spatially explicit model (NSIM, Rosindell & Harmon, 2013) predicts patterns as islands approach equilibrium conditions, in the classic sense of the equilibrium theory of island biogeography (ETIB, MacArthur & Wilson, 1967), reflected in immigration rates, extinction rates and species abundance distributions (SADs).

Within islands, HTS barcoding also harbours the potential to overcome the Wallacean shortfall for arthropod faunas, which is defined as the lack of knowledge regarding the geographical distributions of species (Hortal et al., 2015; Lomolino, 2004). Arjona et al. (2022) provide a clear example of this, revealing substantial improvements to beetle species distribution data from wocDNA metabarcoding. Geographical distribution data for insular arthropod faunas, together with genetic and abundance data at the community-level (also derivable with the HTS barcoding tools) offer an ideal setting to explore linkages between species rarity (geographical, habitat specificity and/or local population size) and endemism (e.g. Fernandez-Palacios et al., 2021; Ribeiro et al., 2005).

5. Biotic interactions of insular arthropods

Islands are providing important advances in the application of ecological network approaches for the understanding of ecosystem function, and vulnerability to disturbance (Traveset et al., 2016). The time-consuming task of arthropod sorting and identification that is needed to quantify plant-arthropod or arthropod-arthropod associations is a bottleneck for addressing the Eltonian shortfall for arthropods, defined as the lack of knowledge of interactions among species or among groups of species (Hortal et al., 2015). However, progress toward addressing this shortfall can be scaled up with the application of HTS barcoding. WocDNA metabarcode data can be used to estimate ecological/trophic networks through co-occurrence analysis (Bohan et al., 2017). Sampling strategies that yield few arthropod individuals per sampled plant would be most efficiently coupled with multiplex barcoding. However, larger arthropod samples, such as aggregat-

ing arthropod samples by plant species (e.g. Rego et al., 2019; Ribeiro et al., 2005), can be coupled to wocDNA metabarcoding. Recently, environmental DNA metabarcoding from plant material offers promise as an additional tool to recover arthropod-plant interactions (Thomsen & Sigsgaard, 2019). Barcode reference sequences for the taxonomic assignment will be desirable, but even in their absence, ecological networks can still be established with higher-level taxonomic assignment. One largely unresolved challenge for understanding the biotic interactions of many arthropod species is the different biology of the life history stages. The problem is exacerbated by the fact that most arthropod sampling methods favour adults, although the larvae of many species may be important for biotic interactions. An in-depth understanding of the interaction thus often requires multiplex barcoding of adults and larvae in order to establish barcode matches between adult and larval stages (Yeo, Puniamoorthy, Ngiam & Meier, 2018).

Moving from association data to trophic interactions can be integrated within a multiplex barcoding framework, and may be particularly useful to improve predictions within the trophic theory of island biogeography (Gravel, Massol, Canard, Mouillot, & Mouquet, 2011; Holt, 2009). Gut or digestive system metabarcoding from appropriate individual DNA extractions (e.g. whole organism) can be used to characterise both herbivore (e.g. Kitson et al., 2015) and predator diet (e.g. Cuff et al., 2021; Kennedy, Lim, Clavel, Krehenwinkel, & Gillespie, 2019). This can be more challenging in predators due to the issue of co-amplification of predator DNA (see Kennedy et al., 2020 for a review), although this obstacle can be overcome via careful primer design (Krehenwinkel et al., 2019). Advances in the characterisation of arthropod microbiomes offer new dimensions to investigate the dynamics of both ecological success (e.g. invasive species) and vulnerability (e.g. range-restricted endemic species), while also investigating the temporal and spatial dynamics of microbiome evolution (e.g. Leo et al., 2021). For example, independent but geographically coincident patterns of island colonisation and speciation, such as those conforming to the progression rule (Shaw & Gillespie, 2016), can be used to understand potential generalities of microbiome evolution, associated with a history of founder event speciation. As a proof of concept, Armstrong et al. (2022) have explored how the associated microbial communities within a lineage of spiders have changed with colonisation across a chronosequence of volcanoes in Hawaii.

6. Spatial and temporal monitoring to understand declines in and threats to insular arthropods

Evidence is accumulating globally that terrestrial insect abundance and biomass are in decline across multiple regions, habitats and taxa (Hallmann et al., 2017; Wagner, Grames, Forister, Berenbaum, & Stopak, 2021), although the historical scarcity of highly-standardised long-term arthropod monitoring programs leaves uncertainty of the nature, scope, and taxonomic and geographic variation of the problem. The establishment of such programs has been limited by the time and specialised expertise required to process samples across whole arthropod communities. It is difficult to evaluate which of the potential drivers of long-term declines are most responsible, and thus most important to address, without extensive data with minimal biases (Cardoso & Leather 2019; van Klink et al., 2020, 2022). Thus, HTS combined with methods to measure abundance provide a route to the practical and comparable long-term monitoring of communities globally.

Islands should be particular priorities for monitoring given their relative biodiversity value, high human impact, and utility as harbingers of more general global change (Fernandez-Palacios et al. 2021). However, they are underrepresented in global initiatives for biodiversity monitoring and biodiversity indicator frameworks, prompting calls for coordinated surveying and monitoring of island biotas (Borges et al., 2018). Two facets of island biota provide cause for concern in the context of potential decline in abundance and biomass. First, general decreases in arthropod abundance are likely to exacerbate extinction risk at the level of individual species, due to already geographically limited range sizes (Manes et al., 2021; Veron, Mouchet, et al., 2019). Second, island biotas are inherently at risk from species invasion and decreased local abundance of native species could increase this threat (Bellard et al., 2017; Russell & Kueffer, 2019; Borges et al., 2020). Understanding island arthropod decline in the (broadly common) absence of historical data (but see e.g. Colom, Traveset, Carreras, & Stefanescu, 2021; Theng et al., 2020) can, to some extent, be addressed by sampling across gradients for suspected drivers of decline, such as climate (Ferreira et al., 2016) and disturbance (Cardoso, Rigal, Fattorini, Terzopoulou, & Borges, 2013). Given the critical focus on abundance and biomass,

multiplex barcoding can be used, and total abundance partitioned to individual species for the identification of more nuanced abundance changes across species. Alternatively, PCR free metagenomic approaches allow estimating the abundance of different arthropod species (Ji et al. 2020), and wocDNA metabarcoding has been demonstrated to reveal relative abundances of species, when coupled with specimen counts (Lim et al. 2021). Although still in early development, CNNs and DL (see above) hold promise for photographing and archiving samples prior to wocDNA metabarcoding, for future abundance estimation (Arribas et al., 2022).

The high throughput and efficiency of HTS barcoding approaches represent a viable long-term solution for monitoring and documenting change within island arthropod communities, and in the context of an iGON, these can be integrated within existing frameworks (e.g. Borges et al., 2018). Suggestions for a coordinated approach to inventory and temporal monitoring, through spatially extensive inventory with a subset of sites subject to temporal sampling (Arribas et al., 2021a), can provide needed baseline data for conservation planning. Range size is frequently used in conservation planning, within which species with small ranges and often declining abundances are given higher priority. Indeed, range restriction and population trends are integral to the International Union for Conservation of Nature (IUCN) criteria to identify and classify species threatened with global extinction. While downstream bottlenecks of the red-listing process are now being addressed by semi-automated systems (Cazalis et al. 2022), upstream the common data deficiency for arthropods within islands limits effective red listing by the IUCN. Strategically designed spatial and temporal HTS barcode sampling networks can provide species records at scales appropriate for both IUCN needs, and the needs of local conservation and management agencies and stakeholders. Such scales go as far as the microhabitat level, as threat might be influenced by it. As an example, classical sampling of Madeiran spiders suggests ground associated species are at greater risk of local extinction than those from canopy microhabitats (Cardoso, Crespo, Silva, Borges, & Boieiro, 2017; Crespo, Silva, Enguidanos, Cardoso, & Arnedo, 2021). This parallels more general inferences for greater climate change impacts for forest floor arthropod species compared to canopy species in Puerto Rico (Lister & Garcia, 2018). More involved implementations of stratigraphically structured sampling and HTS barcoding with abundance data (i.e. multiplex barcoding or wocDNA barcoding with artificial intelligence for image recognition) also has the potential to simultaneously contribute local species records, basic niche information (stratigraphic distribution), and local abundance.

7. Invasive arthropod species detection, identification and implications for island biodiversity

Non-native arthropod species within insular environments represent a fundamental dimension of the ongoing biodiversity crisis (Borges, Rigal, Ros-Prieto, & Cardoso, 2020). The typically depauperate biotas of islands contribute to increased sensitivity to invasive species when compared with continental areas (Bellard et al., 2017), and it is generally understood that early warning and rapid response to new arrivals is necessary to head off establishment and spread, highlighting a need for robust and rapid island monitoring (Borges et al., 2018). A strong argument for the continued effort toward global arthropod barcoding reference databases is the added value these can provide for HTS-based approaches for the biosurveillance of non-indigenous species. Traditional detection methods are expensive, prone to time lags, and require specialised expertise, creating a need for rapid and accurate biosurveillance tools, tailored to the needs of particular biogeographic regions (Westfall, Therriault, & Abbott, 2020). As reference barcode sequences accumulate globally for both recognised and potential arthropod pest species, HTS barcoding for biosurveillance becomes a more powerful alternative to traditional approaches. Even in the absence of reference sequences for taxonomic assignment, genetic signatures can be leveraged for the inference of probable non-native species. Using insects and spiders on the island of Moorea, Andersen et al. (2019) have demonstrated a novel approach to categorise species as being either likely native or likely non-native, based solely on measures of nucleotide diversity. When coupled with spatially structured and temporally replicated haplotype-level wocDNA metabarcoding, novel appearance and increasing site occupancy data could also potentially be leveraged to infer novel non-native species and range expansions. The advent of high-throughput multiplex HTS barcoding also allows for testing the resilience of natural habitats against invasive species. Baloglu et al. (2018) showed that the rich chironomid midge fauna (ca. 300 spp) of a very small remnant of a swamp forest (90 ha) was resilient against invasion by ca. 50 species of “reservoir” chironomid midge species from three adjacent man made reservoirs: only 8 species accounting for ca. 3% of the 14,000 barcoded specimens were shared.

Well-inventoried island systems can be used to test fundamental invasive species theory (Schaefer, Hardy, Silva, Barraclough, & Savolainen, 2011) and, when coupled with temporal sampling, the dynamics of introduced arthropod species abundances can be used to guide management strategy (Matthews, Sadler, Carvalho, Nunes, & Borges, 2019). The often relatively simplified nature of island ecosystems provides opportunities for both island-wide and community-level sampling to contribute to a more general understanding of the properties and dynamics of introduced and invasive species (Borges et al., 2020). Schaefer et al. (2011) sampled the entire Azorean flora for a phylogenetic understanding of evolutionary relatedness as a predictor of invasion potential. In concert with mitogenome backbone trees (see section 1 above), similar opportunities arise for arthropod fractions of island biodiversity with HTS barcode data. Indeed, if combined with DNA sequence-based frameworks to assign likelihood for native or non-native species status, such as that of Andersen et al. (2019), spatially explicit HTS barcode data can address the uncertainty of species status for more robust inferences. Detailed sampling of arthropods to quantify functional trait structure in the Azores has revealed that, in agricultural landscapes, non-native species may contribute positively to the maintenance of some ecosystem functions (Rigal et al., 2018; Ferrante et al., 2022). Barcode reference libraries with relevant trait data, together with HTS barcoding of arthropods across comparable natural and agricultural gradients in other islands, provide a cost effective and logistically feasible pathway to assess the broader generality of these findings.

Conclusions

Advances in high throughput barcoding, together with progress in the field of automated image-based identification, are providing new ways to generate fundamental biodiversity data for arthropods. These exciting developments can be leveraged to address key data shortfalls for arthropods that have important implications for both conservation and management, and more applied ecological and evolutionary understanding. We have focused on islands, due to their biological importance and conservation concern, to assess how such developments can be integrated to advance the understanding, management and conservation of their biotas. Taken together, there is a strong rationale for global, coordinated and funded networks of island Genomic Observatories, to complement other forms of space and ground-based Earth observation. These "biodiversity weather stations" could help monitor and understand climate and degradation-driven biodiversity trends, track the global spread of invasive species in real time, and be harbingers for changes that will ultimately manifest in continental systems. We conclude that high throughput barcoding can be applied to address multiple dimensions of existing data shortfalls for insular arthropods, and that ongoing developments in the area of image-based identification will likely lead to even higher efficiency. The DNA barcode provides a universal currency for measuring and comparing arthropod biodiversity and, if implemented within the framework of an island Genomic Observatories Network, can connect island biodiversity research at a global scale.

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Author contributions

Carmelo Andújar, Paula Arribas and Brent Emerson organised and coordinated the working group. All authors contributed ideas and discussion to this Opinion article. Brent Emerson led the writing, together with Paula Arribas and Carmelo Andújar, with contributions from all authors.

Data Accessibility Statement

No data were analysed.

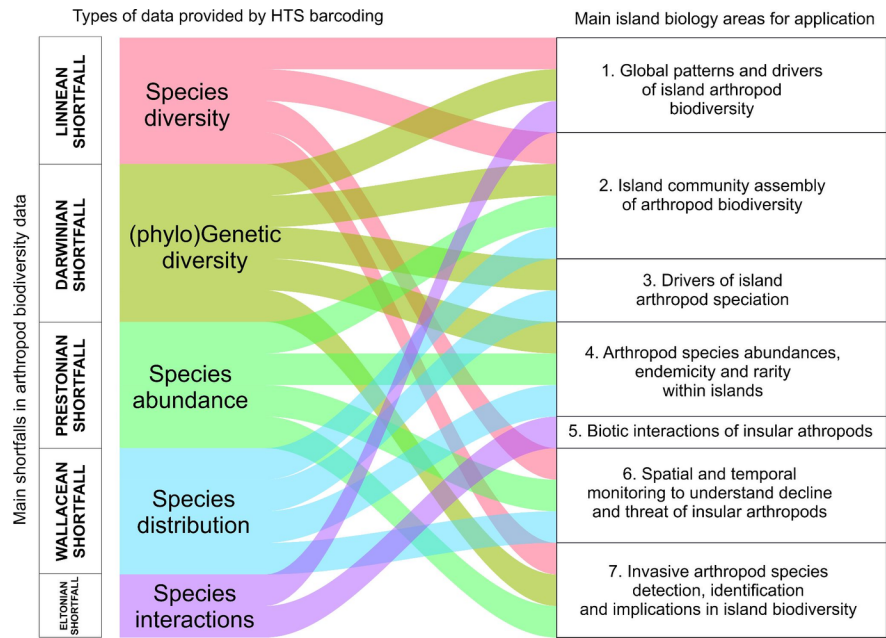


Figure 1. Schematic representation of the types of data provided by HTS barcoding, and their correspondence with key shortfalls for arthropod biodiversity data, and research areas within which collective and harmonised efforts in HTS arthropod inventory could yield significant advances in island biodiversity research.

Table 1. A non-exhaustive list of open questions in island biodiversity research for which HTS arthropod inventory could yield significant advances.

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|--|--|
| 1. Global patterns and drivers of island arthropod biodiversity | How does island biodiversity vary across the globe? To what extent is island biodiversity determined by biogeographic processes? Is there a global pattern of island biodiversity? How do island biodiversity patterns vary across the globe? What are the drivers of island biodiversity? What is the role of island biodiversity in global biodiversity? How do island biodiversity patterns vary across the globe? What metrics are used to measure island biodiversity? What factors influence island biodiversity? How can we conserve island biodiversity? What are the threats to island biodiversity? What species are found on islands? How and why do island species differ from mainland species? What are the evolutionary processes that shape island biodiversity? What role do island biodiversity patterns play in understanding global biodiversity? How do island biodiversity patterns vary across the globe? How, if at all, is island biodiversity changing? Which islands have the highest biodiversity? Which islands have the lowest biodiversity? What is the impact of human activities on island biodiversity? What are the conservation implications of island biodiversity? What are the research priorities for island biodiversity? |
| 2. Island community assembly of arthropod biodiversity | |
| 3. Drivers of island arthropod speciation | |
| 4. Arthropod species abundances, endemism and rarity within islands | |
| 5. Biotic interactions of insular arthropods | |
| 6. Spatial and temporal monitoring to understand declines in and threats to insular arthropods | |
| 7. Invasive arthropod species detection, identification and implications for island biodiversity | |

