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REVIEW

Molecular tools for coral reef restoration: Beyond biomarker discovery

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Abstract

As coral reefs continue to decline due to climate change and other stressors, scientists have proposed adopting genomic tools, such as biomarkers, to aid in the conservation and restoration of these threatened ecosystems. Biomarkers are easily measured indicators of biological processes that can be used to predict or diagnose health, resilience, and other key performance metrics. The ultimate goal of developing biomarkers is to determine the conservation value and utility of a given coral colony, including the host animal, its algal symbionts, and their microbial partners. However, this goal remains distant because most efforts have not yet moved beyond the initial discovery phase. We review recent progress in the development of coral molecular biomarkers from a practical standpoint and consider the many challenges that remain as roadblocks to large-scale implementation. We caution practitioners that, while biomarkers are a promising technology, they are unlikely to be available for field application in the near future barring a rapid shift in research focus from discovery to subsequent validation and field trials. To facilitate such a shift, we propose a stepwise framework to guide additional study in this area, with the aim of accelerating practical molecular biomarker development to enhance coral restoration practice.

KEYWORDS

climate change, coral, genetic management, molecular biomarker, population enhancement

1 | INTRODUCTION

The management of reef-building coral populations has progressed beyond basic conservation to active restoration. This practice is especially evident in Florida and the Caribbean, where multiple in-water nurseries specializing in the asexual propagation of multiple species, particularly the endangered staghorn coral, *Acropora cervicornis*, have been established (Lirman & Schopmeyer 2016; Young, Schopmeyer, & Lirman, 2012). In the last several decades, significant progress has been made in coral husbandry for restoration applications (e.g., Rinkevich, 1995). Currently, practitioners are capable of generating tens of thousands of colonies via microfragmentation in a matter of months, and of managing the in-water grow-out of similar numbers using submerged buoyant structures (Figure 1). One rate-limiting step impeding broader

FIGURE 1 Submerged buoyant structures for the grow-out of *Acropora cervicornis* fragments. Photo credit: Erich Bartels

restoration goals is the outplanting of nursery-reared stock back onto natural reefs. Growth and survival of outplants is highly variable, both among genotypes and reef sites (Bowden-Kerby, 2008; Drury, Manzello, & Lirman, 2017; Lirman et al., 2014), and practitioners currently have no way of reliably matching source corals with their optimum outplant destinations. Moreover, outplanting success does not necessarily equate to restoration of ecological function (Ladd, Burkepile, & Shantz, 2019)

Although maximizing genetic diversity of restored coral populations is paramount (Baums et al., 2019), there is also a growing desire to identify and outplant the most resilient corals, such as those less susceptible to disease outbreaks or more tolerant of temperature stress (e.g., van Oppen, Oliver, Putnam, & Gates, 2015). One impediment to achieving this goal is determining what readily quantifiable phenotypes are most indicative of resilience (for more detailed consideration, see Baums et al., 2019). These restoration priorities have also spurred renewed interest in the development of simplistic assays, such as biomarkers (Box 1), which could provide managers with additional information to aid in outplant design. Recent advances in biotechnology, genomics, and computational power have only increased our ability to identify putative biomarkers (Evans & Hofmann 2012; Sgrò, Lowe, & Hoffmann, 2011; Traylor-Knowles & Palumbi 2014). One hope is that these advances can facilitate rapid identification of resilient corals, diagnose stress events, and provide predictive information to optimize outplanting strategies aimed at preserving genetic diversity and enhancing ecosystem structure and function. A more realistic expectation is that biomarkers may complement other tools and approaches for managing diverse populations to ensure adaptive capacity (Baums et al., 2019). Practitioners recognize this utility, and have been working for years with the scientific community to develop both phenotypic and genomic databases for colonies in the wild, in nurseries, and in outplant projects (Kitchen et al., 2018). For example, the nonprofit Coral Restoration Foundation—the largest operation in the USA—has invested heavily in generating molecular markers to better understand population structure, genetic diversity, and the link between genetic and phenotypic traits in restored populations, a push that was made in part to enhance biomarker discovery (Scott Winters, CEO, pers. comm.). Our goal here is to synthesize the current state of the science for practitioners, temper some of the high expectations associated with coral biomarker discoveries, and provide a framework to guide future research in this area.

Although the potential utility of molecular biomarkers in diagnosing and predicting health outcomes has long been recognized in the coral restoration science community (Downs, Woodley, Richmond, Lanning, & Owen, 2005; Evans & Hofmann 2012; Traylor-Knowles & Palumbi 2014), research in this area has yet to produce any management-ready tools. A Web of Science search identified 127 papers on the topics of "coral" and "biomarker" from 1997 to 2019 and shows that citations exceeded growth in publications (Kolmogorov–Smirnov $D = 0.78$, $P = 1.5 \times 10^{-06}$, Figure 2; Table S1), suggesting that interest is outpacing primary research on the

Box 1. Defining Biomarkers

In 2001, the NIH-funded Biomarkers Definitions Working Group defined biological markers as objectively measurable indicators of a biological processes (Biomarkers Definitions Working Group, 2001). Markers can be diagnostic, meaning they provide some information with respect to an ongoing condition, or predictive, meaning they provide some information that can be used to make a decision about a potential future outcome. Although the original intent was to standardize studies in the rapidly developing field of personalized medicine, this definition is universal and we adopt it here for the purposes of coral restoration ecology. In this sense, there are biomarkers that are already routinely used in coral science and reef management. Diagnostic markers include PAM fluorometry as a proxy for photosynthetic function (Warner, Lesser, & Ralph, 2010) and the CoralWatch Coral Health Chart as a bleaching indicator (Siebeck et al., 2006). Predictive markers include NOAA's degree heating weeks as an indicator of the likelihood of observing mass coral bleaching (Liu, Strong, & Skirving, 2003). The value of such biomarkers is that, when accurately quantified, they are easily assayable substitutes exhibiting strong correlations with meaningful biological phenotypes.

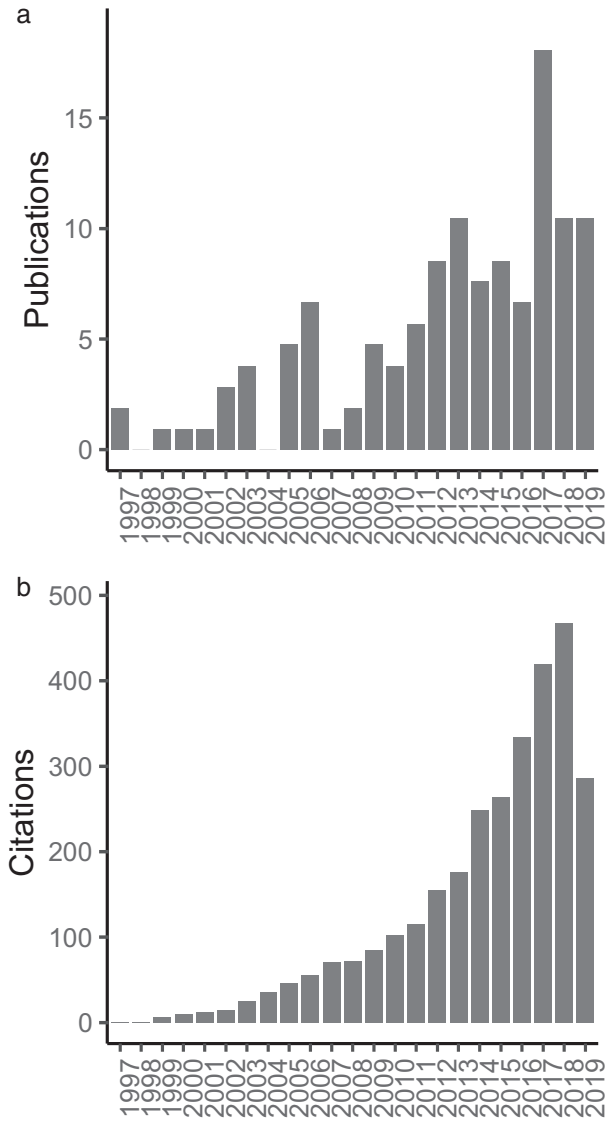

FIGURE 2 Publications on the topic of "coral/s" and "biomarker/s" between 1997 and 2019 (a) total publications by year and (b) sum of times cited by year

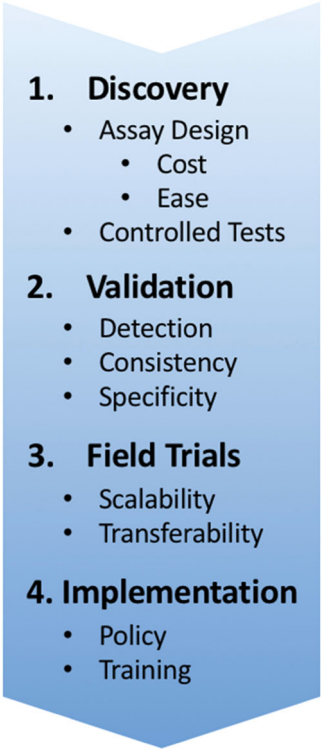

FIGURE 3 Flow chart depicting steps involved in biomarker development (adapted from Willis & Lord 2015)

development of these tools. Similarly, the 13th International Coral Reef Symposium (ICRS) in 2016 featured one session on using genomics for coral reef management,[1] whereas these topics are to be covered by six sessions spread across three themes at the 14th ICRS[2,3,4] in 2020. As is evidenced by numerous studies reporting on putative biomarkers (see below), the barrier lies not in the discovery phase, but in subsequent validation, field trials, and implementation (Traylor-Knowles & Palumbi 2014). There are many steps between identifying a potential biomarker and refining it for standard use, and the inherent difficulties involved in downstream biomarker development and validation are often overlooked. Here, we propose a stepwise research framework for bridging this gap (Figure 3), which is modeled on a similar flow-chart proposed for generating clinical biomarkers (Willis & Lord 2015).

Importantly, we diverge from previous recommendations in that we do not consider understanding the underlying cellular mechanism to be essential in the design of a functional coral biomarker. Instead we follow the approach adopted by the medical field in prioritizing biomarkers according to their ability to robustly predict or diagnose a response (Willis & Lord 2015). Below, we outline each of the proposed steps in detail, explaining the rationale and reviewing relevant literature. Although our discussion is limited to molecular biomarkers, we believe that satisfying the criteria described in this framework will be necessary for developing any type of biomarker, including ecophysiological, environmental, or otherwise.

2 | MOLECULAR BIOMARKER DISCOVERY

Funding for coral restoration fundamentally constrains the type of research undertaken as well as the eventual implementation of newly developed methodology by practitioners. Consequently, it is at this initial stage of biomarker development that researchers should consider the ultimate cost to end-users, both at the level of base cost (for materials and reagents necessary to run the prospective assay) as well as the cost in

person-hours (for sample processing and subsequent analyses necessary to generate usable information). It may be that cutting-edge molecular approaches may not be practical solutions, in the near-term, for restoration programs that operate without access to a molecular laboratory, a stable internet connection, or a computationally trained staff.

The type of assay desired, whether diagnostic or predictive, will also inform initial experimental design and downstream development considerations. As studies focused on human health applications have shown, biomarkers are generally specific to particular conditions and not necessarily transferable, even within the same family of diseases, such as the oncotype test for estrogen receptor-positive breast cancer (Cronin et al., 2007). If true for corals, this may necessitate different markers for each combination of species, trait, and condition, which will further increase costs.

Coral molecular biomarker discovery is not currently a bottleneck: many putative markers have already been proposed (Bay & Palumbi, 2014, 2017; Downs, Mueller, Phillips, Fauth, & Woodley, 2000; Downs, Fauth, et al., 2005; Jin et al., 2016; Kenkel et al., 2011, 2014; Lundgren, Vera, Peplow, Manel, & van Oppen, 2013; Parkinson et al., 2018; Wright et al., 2017). Furthermore, given the substantial progress in the field of coral ecological genomics, additional candidates could be identified through a larger meta-analysis of currently published studies that quantify both 'omic markers and phenotypes of interest. Such an analysis would be facilitated by establishing a broader genotype/phenotype database, similar to those currently available for model organisms (Gramates et al., 2016) and planned for *Acropora cervicornis* and other Caribbean corals (Baums et al., 2019). Because discovery is relatively easy and inexpensive, it should be viewed as an initial step in any applied biomarker project, not an end goal.

2.1 | Cost/benefit analysis

We consider the large-scale restoration of *Acropora cervicornis* in the Florida Keys as a case study in estimating costs from the perspective of practitioners and funding agencies. Here, we quantify direct costs per colony, but other approaches, such as cost per restored hectare, may become more relevant in the future. Since 2007, various organizations have transplanted on the order of 100,000 fragments of *A. cervicornis* along the Florida Reef Tract (Schopmeyer et al., 2017, https://www.coralrestoration.org/restoration), which represents roughly 10,000 fragments per year. At present, major acroporid nurseries in Florida are charging ~$20 per nursery fragment. The total price of this effort (assuming no economies of scale) can therefore be estimated at $10,000–$200,000 in annual coral propagation costs alone. In most cases, the genotypes outplanted at a given site are not native to that site, and are haphazardly selected from the nursery stock, which are in turn sourced from a variety of sites in the area. While average mortality among non-native *A. cervicornis* outplants is ~15% during a typical year (Schopmeyer et al., 2017), it can rise to ~89% in a bleaching year (Drury et al., 2017). Applying this average mortality, and assuming that only 15% of corals would have expired in the absence of heat stress, a single annual bleaching event could represent a loss of $7,400–$148,000.

Rather than outplanting randomly, a hypothetical biomarker to predict thermal tolerance could be used to identify resilient genotypes and prioritize their restoration. If such selection reduced bleaching mortality by even 25% (a reasonable value considering the effect sizes of some commercially important quantitative trait loci (QTLs) in plants; e.g., Anderson, Chao, & Liu, 2007), this would represent a cost saving of $2,225–$44,500 per year, or $22,250–$445,000 per decade. This calculation emphasizes that the cost saving first depends on the price to rear a colony, which likely varies from nursery to nursery, and secondly on marker effectiveness. The cost to outplant a coral fragment varies considerably, with estimates as low as ~$1 USD per unit (Edwards, 2010), to practical examples ranging from ~$5 per unit (Chamberland et al., 2015) up to an extreme ~$150 per unit (Nakamura et al., 2011), depending on the species and scenario. Cost per unit depends both on the input costs (determined by nursery, location, sociocultural factors, species, and productivity) and on survivorship (determined by environment, location, species, and unpredictable disturbance events). If coral propagation costs are in the upper range of the estimate, developing a useful biomarker could be worthwhile, especially considering that the marker could be applied to multiple restoration projects throughout the Caribbean, and that immediate goals envision single teams outplanting 500,000 fragments per year (https://www.xprize.org/visioneering/saving-coral-reefs). At the lower end, however, if coral propagation costs can be minimized, a trial-and-error approach to outplant site selection may indeed be the most cost-effective method, even if it does result in significant subsequent coral mortality.

2.2 | Types of molecular biomarkers: Benefits and barriers

As a consequence of the 'omics revolution, many types of molecular markers can now be evaluated as potential biomarkers in a high-throughput, cost-effective manner. However, before investing in downstream development, the ultimate applicability from the practitioner's perspective should be considered when deciding which methods to explore, as well as the type and quality of information provided by each marker. For example, if a predictive assay is desired, a marker that is a fixed property of an individual may be more desirable

than one that is variable. Below, we summarize different types of biomarkers in the context of these benefits and barriers.

(i) Genetic/genomic biomarkers of host corals are based on associations between DNA variation (among or within individuals, populations, or species) and phenotypes (traits) of interest. These markers range from single nucleotide polymorphisms (SNPs) to repeat variants, such as microsatellites. High-throughput methods used to identify such biomarkers include whole genome/transcriptome sequencing and resequencing, genotyping by sequencing, restriction-site associated DNA (RAD) sequencing, and amplicon sequencing (reviewed by Matz, 2017). One benefit of investing in the downstream development of genomic markers is that many studies aimed at uncovering the genomic basis of adaptive trait variation in corals have already identified putative markers for further development (e.g., Bay & Palumbi, 2014, 2017; Dixon et al., 2015; Jin et al., 2016; Kirk, Howells, Abrego, Burt, & Meyer, 2018; Kitchen et al., 2018; Lundgren et al., 2013). Additionally, DNA sequences are more fixed than any other type of biomarker, and are therefore the most amenable to predictive assays. Barriers to consider include whether assay design can be sufficiently streamlined for general use by restoration practitioners and the transferability of markers when initial discovery studies are focused on non-restoration species.

(ii) Genetic/genomic biomarkers of holobiont community composition are a special case in which the presence/absence or abundance of particular taxonomic units may be associated with metrics of coral host performance and may reflect phenotypes of interest to restoration practitioners. Corals associate with unicellular algal symbionts (Symbiodiniaceae) as well as with other members of the microbial community, such as bacteria (for recent reviews, see Hernandez-Agreda, Leggat, Bongaerts, Herrera, & Ainsworth, 2018; LaJeunesse et al., 2018). The presence/absence or relative abundance of particular Symbiodiniaceae (Bay, Doyle, Logan, & Berkelmans, 2016; Parkinson et al., 2018) and bacteria (Leite et al., 2018; Ziegler, Seneca, Yum, Palumbi, & Voolstra, 2017) have been proposed as potential biomarkers. Specific genomic regions that differentiate taxa, such as 16S or ITS, can be targeted using amplicon sequencing and/or quantitation, such as real-time PCR (e.g., Mieog, van Oppen, Cantin, & Stam, 2007). Alternatively, metagenomics can be used to recover whole genomes and their relative abundances. Biomarkers of holobiont community composition share similar benefits and barriers as host genomic markers, but may not remain fixed during a coral's lifetime. They tend to vary widely among healthy colonies in different environments, and they often respond to stress in stochastic ways (Zaneveld, McMinds, & Vega Thurber, 2017); consequently they may be less useful for developing predictive assays.

(iii) Epigenetic/genomic biomarkers are based on associations between phenotype and different chemical modifications of the genome (rather than changes in DNA sequences themselves). In corals, DNA methylation has received the most attention to date (Dimond & Roberts 2016; Dixon, Bay, & Matz, 2014; Liew et al., 2018). High throughput methods for exploring such markers include whole genome methylation profiling, bisulfite sequencing, and MethylRAD (Kurdyukov & Bullock 2016; Wang et al., 2015). Putative epigenetic biomarkers have been identified in coral hosts (Liew et al., 2018), but may also be present in algal symbionts or other microbial community members. The benefits and barriers of epigenetic biomarkers are similar to genomic markers, but an additional concern is that these biomarkers may change over time and are not necessarily fixed across generations (Heard & Martienssen 2014). Such markers will likely be identified as by-products of an increase in basic research focused on understanding the role of epigenetic modification for phenotypic trait variation in general.

(iv) Gene expression biomarkers are based on associations between phenotypes of interest and changes in mRNA levels (the intermediates that transcribe DNA to protein). Methods used to quantify gene expression patterns include metatranscriptomics, mRNA sequencing, and quantitative reverse transcription PCR (qRT-PCR). Expression biomarkers have progressed furthest in terms of overall development, and have been investigated in the context of both diagnostic and predictive capacities for a variety of phenotypes in the coral host, and to a lesser extent in the algal symbiont (reviewed by Louis, Bhagooli, Kenkel, Baker, & Dyall, 2017). Barriers specific to gene expression biomarkers include the inherent variability of transcription over time and within colonies (Mayfield, Hsiao, Fan, & Chen, 2012; Parkinson et al., 2018); additional work is needed to understand how to control for these variables. Preliminary work has addressed barriers related to transferability and simplicity of assay design (Kenkel et al., 2011; Wright et al., 2017) and further progress in these areas will be facilitated by basic research, especially with high-throughput methods.

(v) Protein-based biomarkers relate levels of specific proteins (the products of gene translation that interact to perform biochemical functions in a cell) to phenotypes of interest. Earlier techniques relied on immunohistochemistry to quantify proteins, but more high-throughput

technologies, such as proteomics, have recently been applied to cnidarians (Oakley et al., 2016, 2017). Protein-based markers have also progressed far in terms of overall marker development (Downs et al., 2000; Downs, Fauth, et al., 2005). However, antibodies needed for immunohistochemistry-based approaches are difficult to standardize across production batches (Baker, 2015) and proteomic methods have yet to catch up to their genomic and transcriptomic counterparts in terms of throughput and repeatability, both for sample preparation and analysis. Similar to gene expression markers, protein levels will likely also vary considerably and marker consistency will be a major consideration during validation studies (Mayfield et al., 2012).

(vi) Metabolomic-based biomarkers focus on associations between levels of metabolites (low molecular weight intermediates and products of enzymatic reactions) and traits of interest. Similar to proteomics, metabolomics methods have lagged behind the other "-omics" approaches in terms of methods development, but are predicted to rise to prominence in clinical biomarker development as methods improve (Monteiro, Carvalho, Bastos, & Guedes de Pinho, 2013). As for all marker types discussed, discovery will be facilitated by basic science research utilizing these techniques, but subsequent development will be hampered by methodological and analytical complexities. Similar to all marker types except genomic, consistency must also be carefully vetted prior to any broadscale implementation.

2.3 | Common experimental design considerations

In many ways, the goals of coral restoration practitioners mirror those of plant breeders, where significantly more research has focused on developing biomarkers to guide management and increase production. Parallel aims include characterizing organismal performance efficiently, choosing which individuals to propagate, and correctly anticipating responses to environmental changes. As technologies have improved, genetically informed plant breeding has adopted several major experimental approaches to develop biomarkers and/or improve performance directly. The types of biomarkers pursued are generally genomic, because breeding programs are inherently predictive. Chronologically, the earliest approaches to identify such predictive markers focused on quantitative trait locus (QTL) mapping, followed by genome-wide association studies (GWAS), then genome-wide selection (GS), and most recently gene editing. The life cycle of each approach tends to comprise periods of initial excitement, followed by a realization of the mismatch between hype and true deliverables, then an acceptance of the reality of what each approach can actually provide (Bernardo, 2016). In recent years, coral biologists have begun to use many of these approaches to address basic research questions.

QTL mapping is an established system for identifying single genes that have a large and consistent effect across many individuals in a population, but one that traditionally requires a known pedigree and several generations to facilitate informative crossing designs. However, many mapping and QTL analysis strategies for plants have been developed more recently to account for their differing propensities to be inbred, their varying generation times, and other factors (Collard, Jahufer, Brouwer, & Pang, 2005; Peace, 2017; Zurn et al., 2018). Most corals have long generation times (e.g., 4 years to reach sexual maturity in fragmented Caribbean acroporids; Chamberland et al., 2016). Long generation times are also observed in crops such as apples and pears, so the QTL mapping strategies used in these systems may be portable to corals (Peace, 2017). Another option to accelerate the utility of a QTL approach is to use alternative cnidarian systems with much shorter generation times and less demanding rearing requirements, such as the upside-down jelly *Cassiopea xamachana* (reviewed by Ohdera et al., 2018).

GWAS designs involve large-scale sequencing of many individuals to identify DNA variation that correlates with phenotypes of interest. They are well suited to coral systems as they typically rely on sampling from large, contemporaneous populations lacking pedigrees. However, GWAS typically recover many genes of small effect, whereas for restoration purposes, few genes of large effect may be more useful (they are typically more predictive, more heritable, and more amenable to cost-effective assays; Bernardo, 2016). It may be possible to improve GWAS-based detection of rare variants by making use of natural selective experiments (e.g., after a major outbreak, the survivors might all be enriched in disease-resistant alleles). Nevertheless, the optimal GWAS design depends on several factors, including sample size, allele frequency, effect size, and genotyping platform (Visscher et al., 2017).

Genome-wide selection (GS) is a new approach in breeding that involves developing thousands of SNP markers combined with extensive phenotyping (often over multiple generations) to predict performance in novel environments, particularly for traits governed by many genes of small effect (Bernardo, 2016). GS has been used successfully in agriculture and animal husbandry (Cabrera-Bosquet, Crossa, von Zitzewitz, Serret, & Araus, 2012; Iwata, Minamikawa, Kajiya-Kanegae, Ishimori, & Hayashi, 2016; Van Eenennaam, Weigel, Young, Cleveland, & Dekkers, 2014), and recent efforts have demonstrated its effectiveness even over single generations (Kumar et al., 2012), suggesting it may soon be feasible in corals. Other cutting-edge technologies such as CRISPR-Cas9 gene editing have been demonstrated in corals (Cleves, Strader, Bay, Pringle, & Matz, 2018), but remain technically

challenging, which make their inclusion in coral restoration programs unlikely in the near future.

3 | MOLECULAR BIOMARKER VALIDATION AND FIELD TRIALS

To progress beyond the discovery phase, putative coral biomarkers must be tested in several ways. Initial results must be validated by additional laboratory studies. The consistency and specificity of the marker should be tested in many individuals and across different time periods and environments. The detection range and limits need to be quantified so that practitioners can be made aware of the level of uncertainty inherent to a particular assay. Although early work has suggested that trade-offs between thermal tolerance and other stress-resistance phenotypes may be minimal (e.g., Muller et al. 2018; Wright et al., 2019), the consequences of marker-assisted selection should also be evaluated. If these small-scale validations yield positive results, trials must then progress beyond controlled laboratory experiments to field-collected samples to see if specificity, consistency, range, and limits remain similar in nature. To our knowledge, only a few sets of coral molecular biomarkers have ever been validated and tested in the field. Here, we review key studies to date.

The first efforts began nearly two decades ago with the development of a suite of protein bioassays to detect host and/or symbiont protein and metabolic condition, oxidative stress response, and xenobiotic response in laboratory-stressed orbicellid colonies (Downs et al., 2000; Downs, Fauth, et al., 2005). Application of these markers to monitor the health of five orbicellid colonies at several reef sites in the Florida Keys seasonally for a year revealed one location exhibiting unique signatures of molecular stress at one time point (Downs, Fauth, et al., 2005). Importantly, the detection of molecular stress preceded a subsequent loss of coral cover at the site, indicating that biomarkers could be used to detect stress and predict coral health outcomes, albeit at low resolution. Field validation was also achieved for additional proteins and early gene expression biomarkers using cDNA microarrays (Edge, Morgan, Gleason, & Snell, 2005; Morgan & Snell 2002; Morgan, Edge, & Snell, 2005).

A more extensive series of laboratory experiments was employed in the development of two "double-gene assays" to discriminate between acute and long-term stress in the Caribbean coral *Porites astreoides* (Kenkel et al., 2011, 2014), which were subsequently validated in four ways. First, field-collected colonies were compared between a high temperature/light inshore site and a low temperature/light offshore site. Consistent with these environmental differences, the acute stress marker indicated that the inshore colonies were somewhat "stressed," whereas offshore colonies were not. Second, corals were sampled in situ during a natural bleaching event. The acute stress marker value was low and indistinguishable in both bleached and healthy colonies, suggesting that the inciting stress had passed, but the long-term stress assay indicated a history of prior stress in only the bleached colonies. Third, an additional laboratory experiment revealed that the acute stress assay also reflected stress levels in the Pacific congener *Porites lobata*. Because of their (relatively) extensive field validation and utility across species, these double-gene stress assays currently show the most promise for broad application as coral molecular biomarkers, although to date they have only been validated in the field during the summer season and for 10 or fewer colonies per treatment.

A more recent field study used a repeated measures design to quantify gene expression responses of 30 colonies of *Acropora cervicornis* exposed to identical thermal stresses at four different time points during the year (Parkinson et al., 2018). While 40% of genes exhibited consistent responses, the remainder varied considerably but were not related to seasonal changes in coral performance (growth, mortality, or bleaching). In one striking example, there was a >1,000-fold difference in the expression of a gene among two independently growing fragments derived from the same donor colony. These results indicate that, while diagnostic markers may be easier to identify than predictive markers, validation nevertheless requires large sample sizes and multiple time points to account for sometimes large transcriptional variation among and within coral colonies.

Two additional studies have incorporated downstream marker validation focusing on predictive assays. The first is the only validation to date of a genomic biomarker (Jin et al., 2016). A GWAS-type design was used to first identify SNPs associated with environmental differences among *Acropora millepora* populations spanning 12° latitude along the Great Barrier Reef. The top two candidate loci were then further validated by comparing corals exhibiting different phenotypic responses to natural stress events. Of 150 colonies sampled across five sites in the Palm Islands group during a natural summer bleaching event, healthy corals exhibited a 12% higher frequency of a particular SNP at one locus. In a subsequent survey of 165 corals following a severe runoff event that increased turbidity and decreased salinity, healthy corals showed a 28% higher SNP frequency of a particular allele at the second locus. Finally, genotypes at the two loci explained a large proportion of the variance in the host coral's coenzyme Q levels and in the algal endosymbiont's photochemistry in response to controlled heat stress, consistent with prior observations. This study illustrates the potential utility of a GWAS design, but additional work is needed to determine the relationship between variance in ecophysiological traits and long-term resilience to disturbance.

In the second study, the gene expression responses of eight *Acropora millepora* colonies to a putative microbial pathogen were investigated (Wright et al., 2017). Two genes were identified whose expression in unexposed fragments predicted survival following bacterial challenge. To validate this two-gene assay and determine its predictive power, an additional 19 colonies were then collected from the field and exposed to a microbial challenge to quantify susceptibility (based on survival). Survival was then related to gene expression in the control treatment samples. The assay successfully categorized the disease risk of a given genotype 73% of the time. As yet, this is the only coral biomarker that can be used on asymptomatic colonies to predict a future health outcome with sufficient resolution to be useful to restoration practitioners.

4 | MOLECULAR BIOMARKER IMPLEMENTATION

So far, no coral molecular biomarkers have been broadly implemented in any conservation programs. Assuming successful discovery, validation, and field trials, there are additional challenges that may limit molecular biomarker adoption when compared to other types of markers. To assay molecular markers, individual colonies must be physically sampled at least once. This is a major bottleneck compared to remote sensing via buoy or satellite, which does not require divers, fueled boats, permits, shipping considerations, or special analytical equipment. Other marker systems, such as the CoralWatch Coral Health Chart (Siebeck, Marshall, Klüter, & Hoegh-Guldberg, 2006), have seen large-scale adoption despite the need for individuals to travel into the field; however, tissue collection requires more training and resources than non-invasive visual inspection or photography, which volunteers can learn rapidly. Such survey methods are relatively inexpensive, whereas molecular techniques always incur additional laboratory and computational costs. Molecular biomarkers are less translatable; it is unlikely that one assay will work across all species of interest, whereas a metric such as color—although requiring species-specific calibration—can be assessed universally. Finally, there is a time component: while well-equipped restoration practitioners could potentially sample a coral and assess a molecular biomarker in just a few days, others in more remote locations may have to wait weeks or months for sample shipment and analysis to be completed.

Nevertheless, molecular biomarkers have been successfully incorporated into restoration and breeding programs for other marine organisms, suggesting they could also be used effectively at large scales to influence health and conservation outcomes for coral reefs. For example, a genomic panel of 188 SNPs was used by wildlife managers to identify introgression from hatchery broodstock into wild salmonid populations in the Pacific Northwest (Steele et al., 2013), and multiple nonacademic laboratories now use genomic data for management of these populations (Garner et al., 2016). On land, larger panels have been incorporated into dairy cattle breeding programs, leading to genetic gains for commercially important traits, improved selection accuracy and breeding value predictions, shorter generation intervals, and reduced costs (reviewed by Pryce & Daetwyler, 2012). Similar results have been reported for plant breeding programs designed for commercial crops such as maize, soybean, rice, and wheat (reviewed by Mammadov, Aggarwal, Buyyarapu, & Kumpatla, 2012). Consequently, although corals present unique challenges, progress in these other fields should guide genomic efforts to restore reefs, and we encourage coral restoration researchers to continue to incorporate these developments into their work.

5 | CONCLUSION

In this review, we take a critical look at the current state of molecular biomarkers for reef restoration and emphasize that broad application is still a distant goal. However, our intent is not to dismiss biomarker research. As our cost/benefit calculations suggest, biomarkers could represent a significant savings per restoration project, and may be useful in situations where a few species have the potential to restore the ecological services a complex reef provides, as may be the case with Caribbean acroporids (but see Ladd et al., 2019). For more complex systems, such as the Great Barrier Reef, it may be worthwhile to focus efforts on select species which play critical roles in recovery, and additional research to identify target species should be prioritized before investing in biomarker development. By highlighting current barriers, we hope to catalyze further research to advance coral molecular biomarker development beyond the discovery phase. Because few studies have attempted the subsequent steps of validation and field trials, no molecular biomarkers are yet ready for implementation, despite broad interest and urgent need. Increased communication between scientists and practitioners will be necessary to determine whether biomarkers are desired and which putative markers should be prioritized for development. Fortunately, as plant and animal breeding programs have shown, implementation can be effective provided researchers, practitioners, and funders are aligned. Given the vast ecological and economic importance of reefs, and the rapid degradation they face in light of climate change, the ideal window for focused biomarker research is now.

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AUTHOR CONTRIBUTIONS

J.E.P. and C.D.K. conceptualized the study; J.E.P. and C.D.K. wrote the original draft; J.E.P., A.C.B., I.B.B., S.W.D., A.G.G., S.A.K., M.V.M, M.W.M., S.R.P., A.A.S., and C.D.K. were associated with reviewing and editing of the manuscript; J.E.P, I.B.B., and C.D.K. were associated with project administration: I.B.B. acquired funding for the study.

[1] 13th International Coral Reef Symposium, *Using Genomics for Coral Reef Management – A Needs Assessment*, https://bit.ly/32r75im

[2] 14th International Coral Reef Symposium, *Scalable Observations and Technologies: What can molecular approaches contribute to determining sublethal stressor effects on coral reefs and evaluating the effectiveness of management interventions?* https://bit.ly/2Lipo48

[3] 14th International Coral Reef Symposium, Conservation and Management: What is the Evidence for and the Future of Resilience-Based Management? https://bit.ly/2XEvsdK

[4] 14th International Coral Reef Symposium, *Interventions and restoration: Can coral climate resilience be enhanced via assisted evolution? How can we leverage advances in evolutionary ecology to maximize the adaptive potential of restored coral populations? What methods and techniques can upscale coral reef restoration? Creating coral reefs in waiting: Can we harness heterogeneity in phenotypic-stress response to optimize coral reef restoration?* https://bit.ly/2Y2hgL1

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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