Mini-Review

Revolutionizing Coral Research: The Power of Holo-Omics

Mohammad M. Binsarhan [1]

[1] Faculty of Marine Science, King Abdul-Aziz University, Jeddah, Saudi Arabia
* Correspondence: sarhan@kau.edu.sa

Abstract: This mini-review discusses the importance of microorganisms in various biological processes and the paradigm shift in researchers' perception of microorganisms' biology and genetics. Microorganisms are now understood to interact with their associated microorganisms, and advanced sequencing technology is used to study these interactions. The article highlights the importance of careful study design to efficiently generate and integrate data derived from meta-omics approaches such as (meta)genomics, (meta)transcriptomics, (meta)proteomics, and (meta)metabolomics to fully explore the extent of interactions between host organisms and their associated microbiota. The article also discusses the usefulness of metabarcoding and metagenomics in studying coral holobiont diversity and composition. While metabarcoding can comprehensively explore the genetic diversity of various biological taxa, it has limitations in classifying prokaryotes based on 16S rRNA amplicons. On the other hand, metagenomics generates vast amounts of short reads sequencing data that can be used to examine microbial diversity with greater precision and predict the possible functions of the gene set that is presented in the sample. However, the ability to link species to their functional capabilities is challenging, especially for microbes with uncertain evolutionary relationships, hidden microbes, and microeukaryotes. Finally, the article highlights the potential usefulness of these approaches in conservation biology and molecular and environmental science.

Keywords: Coral; Holo-omics; Omics; Meta-omics; Genomics.

1. Holo-Omics are the New Trend in Coral Studies

In recent years, there has been a paradigm shift in how researchers perceive the biology and genetics of microorganisms, with a growing recognition of the crucial role of microorganisms in various biological processes. Previously, microorganisms were viewed as isolated genetic entities, but now they are understood as interacting with their associated microorganisms in complex ways. For instance, microorganisms associated with plants and animals are essential for nutrient acquisition [1], immune response [2], development [3], biomolecule synthesis [4], and behavior [5]. Advanced sequencing technology is used to study microorganisms associated with hosts and their interactions, shaping complex organisms' traits. Meta-omic data from both hosts and microbes can reveal these interactions. However, to fully explore the extent of interactions between host organisms and their associated microbiota, it is essential to carefully design studies that can efficiently generate and integrate data derived from meta-omics approaches such as (meta)genomics, (meta)transcriptomics, (meta)proteomics, and (meta)metabolomics [6]. It has become a valuable tool in the study of marine microbes, as these microorganisms are notoriously challenging to cultivate and sustain under laboratory conditions. By leveraging meta-omics techniques, researchers can study marine microbial communities' genetic and functional characteristics without the limitations of traditional culturing methods. This approach allows for a more comprehensive understanding of marine ecosystems and the critical roles played by microbial communities in maintaining ecosystem health and function. The holo-omics approach integrates host and microbiota data to investigate their

 doi:10.20944/preprints202303.0413.v1

interactions. Despite its limitations, it offers opportunities for innovative research in conservation biology and molecular and environmental science.

2. The Promise of Meta-Omics in Unraveling the Coral Holobiont Complexities

Metabarcoding involves utilizing high-throughput sequencing (HTS) to extract sequence information from a complex mixture of genetic material. This information is then compared to a DNA barcode database, which is why it's called metabarcoding [7]. The taxonomic breadth and depth of the detected diversity in a metabarcoding analysis are influenced by the primers' specificity and the reference database used to match genetic sequences to morphological taxonomy [8]. Therefore, the choice of primers and database is crucial for the accurate and comprehensive identification of the species present in the sample. For example, The Tara Oceans project [9–11] utilized advanced Illumina HiSeq technology to sequence a series of recently developed barcodes designed to investigate the abundance and variety of holobiont components such as bacteria and archaea [V4–V5 region of the 16S rRNA] [12], eukaryotes [V9 region of the 18S rRNA] [9], Symbiodiniaceae [ITS2 region of the rDNA] [13–15], and metazoan species [mitochondrial COX1 gene] [16]. This enabled the project to comprehensively explore the genetic diversity of these various biological taxa in coral holobiont. However, the limitation of metabarcoding is that the ability to classify prokaryotes based on 16S rRNA amplicons is mostly restricted to the family or genus level, and it's difficult to classify the species or strain level [17].

Metagenomics is a technique used to examine the composition of biological organisms in a specific habitat. In contrast, genomic studies focus on the genetic material of a particular organism, while metagenomics studies the genetic material of entire communities of organisms. To achieve this, the extracted DNA is subjected to next-generation sequencing (NGS), which generates a vast amount of short reads sequencing data. These short reads can then be assembled to construct a microbial community profile or other valuable information. Then these reads can be used to examine microbial diversity with greater precision than what is achievable through amplicon sequencing [18] by mapping SSU rRNA genes, i.e., 16s and 18s rDNA, to reference databases [19,20] such as SILVA for Prokaryotic [21] and PR2 [22] and Global Ocean DB [23] in addition to predicting the possible functions of the genes set that is presented in the sample [24]. While Matsuo et al., 2021, suggest that it is feasible to identify species using the complete 16S rRNA gene, Jaspers et al., 2019) indicate that it is challenging to link these species to their functional capabilities. It has been suggested that it is possible to predict gene function using 16S rRNA gene data with the aid of available reference genomes [27–30]. Despite the potential usefulness of this approach for well-studied and sequenced microorganisms, Sun et al., 2020, highlight its limited effectiveness for microbes with uncertain evolutionary relationships, hidden microbes, and microeukaryotes. Research on microeukaryote diversity associated with corals has mainly concentrated on disease dynamics, where researchers use microscopy to identify microbes linked to corals. However, this technique has limitations due to its low throughput and targeted nature [32]. The use of non-targeted, culture-independent techniques such as high-throughput metabarcoding has enabled researchers to identify what they were searching for and previously unknown microorganisms in the environment [33]. However, the exploration of the entire micro eukaryotic community is still scarce [34].

The coral meta-omics field is becoming more popular, and many studies have been published using metagenomics approaches and have generated numerous significant hypotheses regarding their roles in stress and bleaching, i.e., Rose et al., 2018, proposed that polygenic evolution is a major factor that influences the differences in ecology and resilience to climate change among corals. However, these hypotheses are mainly based on correlational data. Furthermore, the data are derived from complex datasets that comprise numerous genes and pathways, often making it challenging to pinpoint and fully comprehend the essential constituents of the stress response. Another technical difficulty is

 doi:10.20944/preprints202303.0413.v1

generating data from diverse species without consistent experimental conditions, leading to difficult datasets comparison. One potential way to overcome this technical difficulty is to standardize data processing and analysis across datasets. This could involve developing common protocols for data normalization, quality control, and statistical analysis that are applicable across different coral species and experimental conditions. In addition to the challenge of multi-omics data, the presence of a significant number of genes with unknown functions, or 'dark genes', in corals and algae further complicates the analysis [36]. For instance, in dinoflagellates, approximately one-third of the genes are unannotated, and only a small fraction of these uncharacterized proteins (about 1.4%) have a known domain, making it even more difficult to infer their functions [37]. This lack of functional annotations for these genes hinders our ability to understand the biological processes and pathways that they are involved in and limits the accuracy of our predictions.

Regardless of these limitations, metagenome assembly is a valuable tool for studying microbial populations associated with coral hosts at the genomic level. By generating metagenome-assembled genomes (MAGs) for different microbial species, researchers can investigate the genetic differences between populations of the same species and compare genomes of different species at the amino acid level. This approach can provide insights into the functional roles of coral-associated microbes, their interactions with the host, and their potential contributions to coral health and resilience. Also, it opens doors to compare different assembled genomes at the amino acid level and look for shared gene orthologs. Although this method can be successful in analyzing organisms with small genomes, like prokaryotes, it becomes challenging for eukaryotes because of the complexity of assembling their comparatively larger genomes. Even though the metagenomes assembled from coral samples may not encompass the complete microbial diversity, they offer significant insights into the taxonomy and functional capabilities of many key members of the coral microbiome. Another challenge is the relatively low density of microbial DNA in comparison to the high volume of sequences produced by the coral host. A suggested way to overcome this issue is sequencing deeper and generating more reads to gain more data and consequently obtain more biological information about the coral-associated microorganisms. Another way to avoid the massive read sequencing of the coral host is to design specific metagenomic primers that could prevent or reduce the amplification of the coral host DNA [38] or develop algorithms such as EukRep [39] that can separate the eukaryotic from prokaryotic contigs in metagenomic samples.

3. Meta-Transcriptomics: A Powerful Tool for Studying Coral Holobiont Functionality

Meta-transcriptomics is a powerful tool that not only allows for the analysis of the composition of the microbial community but also sheds light on the active functional profile of the community by identifying the genes that are expressed [40]. It has provided a vast amount of data in coral research, presenting an opportunity to conduct metagenomic investigations aimed at identifying crucial and essential pathways [36]. This information is essential for understanding the role of the microbial community in the coral ecosystem and their interactions with the coral host. The analysis of the holo-transcriptomes of coral holobiont collected from various points along the transect is crucial for gaining genetic insights into ecological and evolutionary queries about both the community and intraspecific levels. Moreover, it gained popularity in the coral omics field, with the utilization of tools such as microarrays and RNA-seq for profiling coral transcriptomes to uncover gene expression patterns related to thermal stress [41].

The identification of differentially expressed genes (DEGs) is allowing researchers to investigate the response to various environmental conditions [42] such as heat stress [43,44] and adaptation to mesophotic conditions [45]. Also, the creation of transcriptome databases has facilitated progress in coral science [46]. Besides, using different protocols for

 doi:10.20944/preprints202303.0413.v1

each domain of the holobiont (Eukaryotic and Prokaryotic) would provide high-resolution results for each holobiont component. For example, use a polyA+ enrichment protocol to obtain eukaryotic mRNA to analyze the coral and Symbiodiniaceae dual transcriptome and remove rRNA from the remaining polyA- fraction to sequence microbial mRNA [47]. Moreover, it is possible to utilize various metatranscriptomics protocols to investigate the gene expression of Cnidaria, Symbiodiniaceae, bacteria, archaea, other microorganisms, and even viruses from the original tissue samples [48,49]. Additionally, researchers have utilized Meta transcriptomics to investigate coral-algal symbiosis [45,50–52], natural bleaching [53–55], and coral diseases [56–59]. This approach has significantly advanced our understanding of coral biology and provided valuable insights into the mechanisms underlying corals' response to thermal stress. [60].

Despite the potential of current meta-transcriptomic techniques, various challenges still prevent their extensive use. One of these obstacles is the high proportion of ribosomal RNA in the collected RNA, which can significantly decrease the quantity of mRNA, the primary target of transcriptomic research. To address this issue, some attempts have been made to efficiently eliminate ribosomal RNA [61]. Second, the instability of mRNA is a well-known issue that can impact the sample's integrity before sequencing [62]. Additionally, distinguishing between host and microbial RNA can be difficult despite the availability of different enrichment kits. However, if the host's reference genome is available, this can also be accomplished computationally, as demonstrated by Pérez-Losada et al., 2015 research on the human airway microbiome and its host-pathogen interactions. Lastly, the coverage of transcriptome reference databases is restricted.

4. Understanding Coral Holobiont through Metabolomic and Proteomics

The field of metabolomics involves identifying all metabolites in a sample to understand their relationship with cellular processes [64]. This approach, in combination with pathways information, can lead to the generation of new hypotheses. The metabolome is believed to be the most reliable indicator of the environment's health and any changes in homeostasis, such as dysbiosis [65]. The production of specific metabolites varies with changes in metabolic activity, making metabolomics a valuable tool for pathway analysis [66]. Furthermore, metabolomics holds promise in drug discovery and pharmacogenomics for personalized medicine [67]. Metabolomics studies have revealed new insights into the interactions within the coral holobiont. For example, platelet activation factors were found to increase at coral-algal interfaces, indicating their role in these interactions [68]. Lipid classes such as betaine-lipids and diacyl-glycerides were correlated with previous coral colonies' bleaching events and can also serve as disease markers [69,70]. Moreover, dipeptides were identified as indicators of heat stress in corals [71]. Various chemical groups comprise these metabolites, and they may significantly impact the microbiome of the surface mucopolysaccharide layer (SML) [72]. One of the metabolites identified is estrogen, which regulates stress responses and modifies the microbiome composition [73,74]. Although meta-transcriptomics has demonstrated its usefulness in the limited cases where it has been utilized, its integration with other meta-omics methodologies can provide additional validation of gene expression patterns, enable quantification of their effects, and offer insight into novel metabolic and symbiotic interactions. Mass-spectrometry-based techniques such as proteomics and metabolomics are revealing groundbreaking discoveries in holobiont research [75].

Although metabolomics is a promising field with lower costs compared to sequencing, it has several limitations [76]. It has been slow to progress due to significant challenges in detecting metabolites present in low abundance and the high number of hidden metabolites commonly found in biological systems [77]. The complexity of chemical formulae and structures poses the biggest challenge in mass spectrometry-based metabolomics, where annotations can only be confirmed reliably with comparisons to known standards [75]. Standardization is necessary for metabolomics due to the impact of extraction protocols

 doi:10.20944/preprints202303.0413.v1

on detected molecules. While some metabolites are prone to degradation, others are not efficiently extracted [76,78]. A significant challenge in the study of the holobiont is connecting metabolites with their respective producing organisms. Although the statistical correlation of metabolite and amplicon sequencing data can aid in this task [79], it is only applicable to identifying unique secondary metabolites. Another key challenge is determining whether a metabolite was produced by the host or microbiome making their identification and assignment to specific organisms more difficult. Additionally, metabolomics results must be combined with other omics data to determine the association between specific genes, enzymes, or pathways with a particular metabolite. Therefore, new approaches that deal with integrated omics are needed to address these challenges [62].

5. Conclusions

The current data about complex ecosystems like coral holobiont is inconclusive, and more investigations are needed. Interestingly, the application of meta-omics methods and advances in computational tools would give access to microbiome functions and help researchers understand how microbiome taxa are interconnected. It is equally important to comprehend the function of unannotated dark genes that may be host-specific innovations or adaptations to environmental conditions. The understanding of coral responses to various stressors is imperative for improving multi-omics protocols and databases for coral studies. This will enable the development of effective conservation and management strategies to protect these valuable ecosystems. In the realm of coral omics, a key objective is to pinpoint the essential genes and species involved in responding to different types of stresses and elucidate how variations in this response correlate with differences in stress resilience. Despite the significant increase in research efforts in the field, the lack of consistency in experimental design, methodologies, and the range of species investigated still poses a considerable challenge in comparing results across studies. It is necessary to develop multi-omics datasets that incorporate standardized protocols for a variety of model coral species reflecting their diversity and create datasets with maximum comparability to enable researchers to identify similarities and differences among the model corals.

References

1. Falcinelli, S., Picchietti, S., Rodiles, A., Cossignani, L., Merrifield, D.L., Taddei, A.R., Maradonna, F., Olivotto, I., Gioacchini, G., and Carnevali, O. (2015). Lactobacillus rhamnosus lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism. Sci Rep *5*, 9336. 10.1038/srep09336.

2. Wu, H.-J., and Wu, E. (2012). The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes *3*, 4–14. 10.4161/gmic.19320.

3. Rudman, S.M., Greenblum, S., Hughes, R.C., Rajpurohit, S., Kiratli, O., Lowder, D.B., Lemmon, S.G., Petrov, D.A., Chaston, J.M., and Schmidt, P. (2019). Microbiome composition shapes rapid genomic adaptation of *Drosophila melanogaster*. Proceedings of the National Academy of Sciences *116*, 20025–20032. 10.1073/pnas.1907787116.

4. Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., and Pettersson, S. (2012). Host-Gut Microbiota Metabolic Interactions. Science (1979) *336*, 1262–1267. 10.1126/science.1223813.

5. Liang, S., Wu, X., and Jin, F. (2018). Gut-Brain Psychology: Rethinking Psychology From the Microbiota–Gut–Brain Axis. Front Integr Neurosci *12*. 10.3389/fnint.2018.00033.

6. Nyholm, L., Koziol, A., Marcos, S., Botnen, A.B., Aizpurua, O., Gopalakrishnan, S., Limborg, M.T., Gilbert, M.T.P., and Alberdi, A. (2020). Holo-Omics: Integrated Host-Microbiota Multi-omics for Basic and Applied Biological Research. iScience *23*, 101414. 10.1016/j.isci.2020.101414.

7. Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D.M., Vere, N., *et al.* (2017). Environmental <scp>DNA</scp> metabarcoding: Transforming how we survey animal and plant communities. Mol Ecol *26*, 5872–5895. 10.1111/mec.14350.

8. Seymour, M. (2022). Environmental DNA Advancing Our Understanding and Conservation of Inland Waters. In Encyclopedia of Inland Waters (Elsevier), pp. 685–698. 10.1016/B978-0-12-819166-8.00070-0.

9. de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C., Le Bescot, N., Probert, I., *et al.* (2015). Eukaryotic plankton diversity in the sunlit ocean. Science (1979) *348*. 10.1126/science.1261605.

10. Decelle, J., Carradec, Q., Pochon, X., Henry, N., Romac, S., Mahé, F., Dunthorn, M., Kourlaiev, A., Voolstra, C.R., Wincker, P., *et al.* (2018). Worldwide Occurrence and Activity of the Reef-Building Coral Symbiont Symbiodinium in the Open Ocean. Current Biology *28*, 3625-3633.e3. 10.1016/j.cub.2018.09.024.

11. Sunagawa, S., Karsenti, E., Bowler, C., and Bork, P. (2015). Computational eco-systems biology in <scp>*T*</scp> *ara* <scp>O</scp> ceans: translating data into knowledge. Mol Syst Biol *11*, 809. 10.15252/msb.20156272.

12. Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., *et al.* (2016). Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. mSystems *1*. 10.1128/mSystems.00009-15.

13. Hume, B.C.C., Ziegler, M., Poulain, J., Pochon, X., Romac, S., Boissin, E., de Vargas, C., Planes, S., Wincker, P., and Voolstra, C.R. (2018). An improved primer set and amplification protocol with increased specificity and sensitivity targeting the *Symbiodinium* ITS2 region. PeerJ *6*, e4816. 10.7717/peerj.4816.

14. Arif, C., Daniels, C., Bayer, T., Banguera-Hinestroza, E., Barbrook, A., Howe, C.J., LaJeunesse, T.C., and Voolstra, C.R. (2014). Assessing <scp>*S*</scp> *ymbiodinium* diversity in scleractinian corals via next-generation sequencing-based genotyping of the ITS2 <scp>rDNA</scp> region. Mol Ecol *23*, 4418–4433. 10.1111/mec.12869.

15. Hume, B.C.C., Smith, E.G., Ziegler, M., Warrington, H.J.M., Burt, J.A., LaJeunesse, T.C., Wiedenmann, J., and Voolstra, C.R. (2019). SymPortal: A novel analytical framework and platform for coral algal symbiont next-generation sequencing *ITS2* profiling. Mol Ecol Resour *19*, 1063–1080. 10.1111/1755-0998.13004.

16. Leray, M., and Knowlton, N. (2015). DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. Proceedings of the National Academy of Sciences *112*, 2076–2081. 10.1073/pnas.1424997112.

17. Johnson, J.S., Spakowicz, D.J., Hong, B.-Y., Petersen, L.M., Demkowicz, P., Chen, L., Leopold, S.R., Hanson, B.M., Agresta, H.O., Gerstein, M., *et al.* (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat Commun *10*, 5029. 10.1038/s41467-019-13036-1.

18. Xu, W., Chen, T., Pei, Y., Guo, H., Li, Z., Yang, Y., Zhang, F., Yu, J., Li, X., Yang, Y., *et al.* (2021). Characterization of Shallow Whole-Metagenome Shotgun Sequencing as a High-Accuracy and Low-Cost Method by Complicated Mock Microbiomes. Front Microbiol *12*. 10.3389/fmicb.2021.678319.

19. Salazar, G., Ruscheweyh, H.-J., Hildebrand, F., Acinas, S.G., and Sunagawa, S. (2021). mTAGs: taxonomic profiling using degenerate consensus reference sequences of ribosomal RNA genes. Bioinformatics *38*, 270–272. 10.1093/bioinformatics/btab465.

20. Vaulot, D., Sim, C.W.H., Ong, D., Teo, B., Biwer, C., Jamy, M., and Lopes dos Santos, A. (2022). <scp> metaPR ^{2} </scp>: A database of eukaryotic <scp>18S rRNA</scp> metabarcodes with an emphasis on protists. Mol Ecol Resour *22*, 3188–3201. 10.1111/1755-0998.13674.

21. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res *41*, D590–D596. 10.1093/nar/gks1219.

22. Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., *et al.* (2012). The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Res *41*, D597–D604. 10.1093/nar/gks1160.

23. Obiol, A., Giner, C.R., Sánchez, P., Duarte, C.M., Acinas, S.G., and Massana, R. (2020). A metagenomic assessment of microbial eukaryotic diversity in the global ocean. Mol Ecol Resour *20*, 718–731. 10.1111/1755-0998.13147.

24. Bashir, Y., Pradeep Singh, S., and Kumar Konwar, B. (2014). Metagenomics: An Application Based Perspective. Chinese Journal of Biology *2014*, 1–7. 10.1155/2014/146030.

25. Matsuo, Y., Komiya, S., Yasumizu, Y., Yasuoka, Y., Mizushima, K., Takagi, T., Kryukov, K., Fukuda, A., Morimoto, Y., Naito, Y., *et al.* (2021). Full-length 16S rRNA gene amplicon analysis of human gut microbiota using MinION™ nanopore sequencing confers species-level resolution. BMC Microbiol *21*, 35. 10.1186/s12866-021-02094-5.

26. Jaspers, C., Fraune, S., Arnold, A.E., Miller, D.J., Bosch, T.C.G., and Voolstra, C.R. (2019). Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches. Zoology *133*, 81–87. 10.1016/j.zool.2019.02.007.

27. D Ainsworth, T., Krause, L., Bridge, T., Torda, G., Raina, J.-B., Zakrzewski, M., Gates, R.D., Padilla-Gamiño, J.L., Spalding, H.L., Smith, C., *et al.* (2015). The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. ISME J *9*, 2261–2274. 10.1038/ismej.2015.39.

28. Ziegler, M., Seneca, F.O., Yum, L.K., Palumbi, S.R., and Voolstra, C.R. (2017). Bacterial community dynamics are linked to patterns of coral heat tolerance. Nat Commun *8*, 14213. 10.1038/ncomms14213.

29. Hernandez-Agreda, A., Leggat, W., Bongaerts, P., Herrera, C., and Ainsworth, T.D. (2018). Rethinking the Coral Microbiome: Simplicity Exists within a Diverse Microbial Biosphere. mBio *9*. 10.1128/mBio.00812-18.

30. Röthig, T., Ochsenkühn, M.A., Roik, A., van der Merwe, R., and Voolstra, C.R. (2016). Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. Mol Ecol *25*, 1308–1323. 10.1111/mec.13567.

31. Sun, S., Jones, R.B., and Fodor, A.A. (2020). Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. Microbiome *8*, 46. 10.1186/s40168-020-00815-y.

32. Bonacolta, A.M., Weiler, B.A., Porta-Fitó, T., Sweet, M., Keeling, P., and del Campo, J. (2023). Beyond the Symbiodiniaceae: diversity and role of microeukaryotic coral symbionts. Coral Reefs. 10.1007/s00338-023-02352-0.

33. TABERLET, P., COISSAC, E., HAJIBABAEI, M., and RIESEBERG, L.H. (2012). Environmental DNA. Mol Ecol *21*, 1789–1793. 10.1111/j.1365-294X.2012.05542.x.

34. Keeling, P.J., and Campo, J. del (2017). Marine Protists Are Not Just Big Bacteria. Current Biology *27*, R541–R549. 10.1016/j.cub.2017.03.075.

35. Rose, N.H., Bay, R.A., Morikawa, M.K., and Palumbi, S.R. (2018). Polygenic evolution drives species divergence and climate adaptation in corals. Evolution (N Y) *72*, 82–94. 10.1111/evo.13385.

36. Cleves, P.A., Shumaker, A., Lee, J., Putnam, H.M., and Bhattacharya, D. (2020). Unknown to Known: Advancing Knowledge of Coral Gene Function. Trends in Genetics *36*, 93–104. 10.1016/j.tig.2019.11.001.

37. Stephens, T.G., Ragan, M.A., Bhattacharya, D., and Chan, C.X. (2018). Core genes in diverse dinoflagellate lineages include a wealth of conserved dark genes with unknown functions. Sci Rep *8*, 17175. 10.1038/s41598-018-35620-z.

38. Tan, S., and Liu, H. (2018). Unravel the hidden protistan diversity: application of blocking primers to suppress PCR amplification of metazoan DNA. Appl Microbiol Biotechnol *102*, 389–401. 10.1007/s00253-017-8565-1.

39. West, P.T., Probst, A.J., Grigoriev, I. v., Thomas, B.C., and Banfield, J.F. (2018). Genome-reconstruction for eukaryotes from complex natural microbial communities. Genome Res *28*, 569–580. 10.1101/gr.228429.117.

40. Moran, M.A. (2009). Metatranscriptomics: Eavesdropping on Complex Microbial Communities. Microbe Magazine *4*, 329–335. 10.1128/microbe.4.329.1.

41. Cziesielski, M.J., Schmidt-Roach, S., and Aranda, M. (2019). The past, present, and future of coral heat stress studies. Ecol Evol *9*, 10055–10066. 10.1002/ece3.5576.

42. Hrdlickova, R., Toloue, M., and Tian, B. (2017). <scp>RNA</scp> -Seq methods for transcriptome analysis. WIREs RNA *8*. 10.1002/wrna.1364.

43. Voolstra, C.R., Valenzuela, J.J., Turkarslan, S., Cárdenas, A., Hume, B.C.C., Perna, G., Buitrago-López, C., Rowe, K., Orellana, M. V., Baliga, N.S., *et al.* (2021). Contrasting heat stress response patterns of coral holobionts across the Red Sea suggest distinct mechanisms of thermal tolerance. Mol Ecol *30*, 4466–4480. 10.1111/mec.16064.

44. Savary, R., Barshis, D.J., Voolstra, C.R., Cárdenas, A., Evensen, N.R., Banc-Prandi, G., Fine, M., and Meibom, A. (2021). Fast and pervasive transcriptomic resilience and acclimation of extremely heat-tolerant coral holobionts from the northern Red Sea. Proceedings of the National Academy of Sciences *118*. 10.1073/pnas.2023298118.

45. Yum, L.K., Baumgarten, S., Röthig, T., Roder, C., Roik, A., Michell, C., and Voolstra, C.R. (2017). Transcriptomes and expression profiling of deep-sea corals from the Red Sea provide insight into the biology of azooxanthellate corals. Sci Rep *7*, 6442. 10.1038/s41598-017-05572-x.

46. Zhang, Y., Chen, Q., Xie, J.Y., Yeung, Y.H., Xiao, B., Liao, B., Xu, J., and Qiu, J.-W. (2019). Development of a transcriptomic database for 14 species of scleractinian corals. BMC Genomics *20*, 387. 10.1186/s12864-019-5744-8.

47. Planes, S., Allemand, D., Agostini, S., Banaigs, B., Boissin, E., Boss, E., Bourdin, G., Bowler, C., Douville, E., Flores, J.M., *et al.* (2019). The Tara Pacific expedition—A pan-ecosystemic approach of the "-omics" complexity of coral reef holobionts across the Pacific Ocean. PLoS Biol *17*, e3000483. 10.1371/journal.pbio.3000483.

48. Carradec, Q., Pelletier, E., Da Silva, C., Alberti, A., Seeleuthner, Y., Blanc-Mathieu, R., Lima-Mendez, G., Rocha, F., Tirichine, L., Labadie, K., *et al.* (2018). A global ocean atlas of eukaryotic genes. Nat Commun *9*, 373. 10.1038/s41467-017-02342-1.

49. Alberti, A., Poulain, J., Engelen, S., Labadie, K., Romac, S., Ferrera, I., Albini, G., Aury, J.-M., Belser, C., Bertrand, A., *et al.* (2017). Viral to metazoan marine plankton nucleotide sequences from the Tara Oceans expedition. Sci Data *4*, 170093. 10.1038/sdata.2017.93.

50. Mohamed, A.R., Andrade, N., Moya, A., Chan, C.X., Negri, A.P., Bourne, D.G., Ying, H., Ball, E.E., and Miller, D.J. (2020). Dual RNA-sequencing analyses of a coral and its native symbiont during the establishment of symbiosis. Mol Ecol *29*, 3921–3937. 10.1111/mec.15612.

51. Yuyama, I., Ishikawa, M., Nozawa, M., Yoshida, M., and Ikeo, K. (2018). Transcriptomic changes with increasing algal symbiont reveal the detailed process underlying establishment of coral-algal symbiosis. Sci Rep *8*, 16802. 10.1038/s41598-018-34575-5.

52. Mohamed, A.R., Cumbo, V., Harii, S., Shinzato, C., Chan, C.X., Ragan, M.A., Bourne, D.G., Willis, B.L., Ball, E.E., Satoh, N., *et al.* (2016). The transcriptomic response of the coral *Acropora digitifera* to a competent *Symbiodinium* strain: the symbiosome as an arrested early phagosome. Mol Ecol *25*, 3127–3141. 10.1111/mec.13659.

53. Pinzón, J.H., Kamel, B., Burge, C.A., Harvell, C.D., Medina, M., Weil, E., and Mydlarz, L.D. (2015). Whole transcriptome analysis reveals changes in expression of immune-related genes during and after bleaching in a reef-building coral. R Soc Open Sci *2*, 140214. 10.1098/rsos.140214.

54. Rose, N.H., Seneca, F.O., and Palumbi, S.R. (2016). Gene Networks in the Wild: Identifying Transcriptional Modules that Mediate Coral Resistance to Experimental Heat Stress. Genome Biol Evol *8*, 243–252. 10.1093/gbe/evv258.

55. Seneca, F.O., and Palumbi, S.R. (2015). The role of transcriptome resilience in resistance of corals to bleaching. Mol Ecol *24*, 1467–1484. 10.1111/mec.13125.

56. Frazier, M., Helmkampf, M., Bellinger, M.R., Geib, S.M., and Takabayashi, M. (2017). De novo metatranscriptome assembly and coral gene expression profile of Montipora capitata with growth anomaly. BMC Genomics *18*, 710. 10.1186/s12864-017-4090-y.

57. Anderson, D.A., Walz, M.E., Weil, E., Tonellato, P., and Smith, M.C. (2016). RNA-Seq of the Caribbean reef-building coral *Orbicella faveolata* (Scleractinia-Merulinidae) under bleaching and disease stress expands models of coral innate immunity. PeerJ *4*, e1616. 10.7717/peerj.1616.

58. Wright, R.M., Aglyamova, G. V, Meyer, E., and Matz, M. V (2015). Gene expression associated with white syndromes in a reef building coral, Acropora hyacinthus. BMC Genomics *16*, 371. 10.1186/s12864-015-1540-2.

59. Libro, S., Kaluziak, S.T., and Vollmer, S. V. (2013). RNA-seq Profiles of Immune Related Genes in the Staghorn Coral Acropora cervicornis Infected with White Band Disease. PLoS One *8*, e81821. 10.1371/journal.pone.0081821.

60. Barshis, D.J., Ladner, J.T., Oliver, T.A., Seneca, F.O., Traylor-Knowles, N., and Palumbi, S.R. (2013). Genomic basis for coral resilience to climate change. Proceedings of the National Academy of Sciences *110*, 1387–1392. 10.1073/pnas.1210224110.

61. Peano, C., Pietrelli, A., Consolandi, C., Rossi, E., Petiti, L., Tagliabue, L., De Bellis, G., and Landini, P. (2013). An efficient rRNA removal method for RNA sequencing in GC-rich bacteria. Microb Inform Exp *3*, 1. 10.1186/2042-5783-3-1.

62. Aguiar-Pulido, V., Huang, W., Suarez-Ulloa, V., Cickovski, T., Mathee, K., and Narasimhan, G. (2016). Metagenomics, Metatranscriptomics, and Metabolomics Approaches for Microbiome Analysis. Evolutionary Bioinformatics *12s1*, EBO.S36436. 10.4137/EBO.S36436.

63. Pérez-Losada, M., Castro-Nallar, E., Bendall, M.L., Freishtat, R.J., and Crandall, K.A. (2015). Dual Transcriptomic Profiling of Host and Microbiota during Health and Disease in Pediatric Asthma. PLoS One *10*, e0131819. 10.1371/journal.pone.0131819.

64. Fiehn, O. (2002). Metabolomics--the link between genotypes and phenotypes. Plant Mol Biol *48*, 155–71.

65. Bernini, P., Bertini, I., Luchinat, C., Nepi, S., Saccenti, E., Schäfer, H., Schütz, B., Spraul, M., and Tenori, L. (2009). Individual Human Phenotypes in Metabolic Space and Time. J Proteome Res *8*, 4264–4271. 10.1021/pr900344m.

66. Krumsiek, J., Mittelstrass, K., Do, K.T., Stückler, F., Ried, J., Adamski, J., Peters, A., Illig, T., Kronenberg, F., Friedrich, N., *et al.* (2015). Gender-specific pathway differences in the human serum metabolome. Metabolomics *11*, 1815–1833. 10.1007/s11306-015-0829-0.

67. Mastrangelo, A., Armitage, E., García, A., and Barbas, C. (2015). Metabolomics as a Tool for Drug Discovery and Personalised Medicine. A Review. Curr Top Med Chem *14*, 2627–2636. 10.2174/1568026614666141215124956.

68. Quinn, R.A., Vermeij, M.J.A., Hartmann, A.C., Galtier d'Auriac, I., Benler, S., Haas, A., Quistad, S.D., Lim, Y.W., Little, M., Sandin, S., *et al.* (2016). Metabolomics of reef benthic interactions reveals a bioactive lipid involved in coral defence. Proceedings of the Royal Society B: Biological Sciences *283*, 20160469. 10.1098/rspb.2016.0469.

69. Deutsch, J.M., Jaiyesimi, O.A., Pitts, K.A., Houk, J., Ushijima, B., Walker, B.K., Paul, V.J., and Garg, N. (2021). Metabolomics of Healthy and Stony Coral Tissue Loss Disease Affected Montastraea cavernosa Corals. Front Mar Sci *8*. 10.3389/fmars.2021.714778.

70. Roach, T.N.F., Little, M., Arts, M.G.I., Huckeba, J., Haas, A.F., George, E.E., Quinn, R.A., Cobián-Güemes, A.G., Naliboff, D.S., Silveira, C.B., *et al.* (2020). A multiomic analysis of in situ coral–turf algal interactions. Proceedings of the National Academy of Sciences *117*, 13588–13595. 10.1073/pnas.1915455117.

71. Williams, A., Chiles, E.N., Conetta, D., Pathmanathan, J.S., Cleves, P.A., Putnam, H.M., Su, X., and Bhattacharya, D. (2021). Metabolomic shifts associated with heat stress in coral holobionts. Sci Adv *7*. 10.1126/sciadv.abd4210.

72. Ochsenkühn, M.A., Schmitt-Kopplin, P., Harir, M., and Amin, S.A. (2018). Coral metabolite gradients affect microbial community structures and act as a disease cue. Commun Biol *1*, 184. 10.1038/s42003-018-0189-1.

73. Vilela, C.L.S., Villela, H.D.M., Duarte, G.A.S., Santoro, E.P., Rachid, C.T.C.C., and Peixoto, R.S. (2021). Estrogen induces shift in abundances of specific groups of the coral microbiome. Sci Rep *11*, 2767. 10.1038/s41598-021-82387-x.

74. Stien, D., Suzuki, M., Rodrigues, A.M.S., Yvin, M., Clergeaud, F., Thorel, E., and Lebaron, P. (2020). A unique approach to monitor stress in coral exposed to emerging pollutants. Sci Rep *10*, 9601. 10.1038/s41598-020-66117-3.

75. Mohamed, A.R., Ochsenkühn, M.A., Kazlak, A., Moustafa, A., and Amin, S.A. (2023). The coral microbiome: Towards an understanding of the molecular mechanisms of coral-microbiota interactions. FEMS Microbiol Rev. 10.1093/femsre/fuad005.

76. Lu, Y., Jiang, J., Zhao, H., Han, X., Xiang, Y., and Zhou, W. (2020). Clade-Specific Sterol Metabolites in Dinoflagellate Endosymbionts Are Associated with Coral Bleaching in Response to Environmental Cues. mSystems *5*. 10.1128/mSystems.00765-20.

77. Vohsen, S.A., Fisher, C.R., and Baums, I.B. (2019). Metabolomic richness and fingerprints of deep-sea coral species and populations. Metabolomics *15*, 34. 10.1007/s11306-019-1500-y.

78. Andersson, E.R., Day, R.D., Loewenstein, J.M., Woodley, C.M., and Schock, T.B. (2019). Evaluation of Sample Preparation Methods for the Analysis of Reef-Building Corals Using ^{1}H-NMR-Based Metabolomics. Metabolites *9*. 10.3390/metabo9020032.

79. Jorissen, H., Galand, P.E., Bonnard, I., Meiling, S., Raviglione, D., Meistertzheim, A.-L., Hédouin, L., Banaigs, B., Payri, C.E., and Nugues, M.M. (2021). Coral larval settlement preferences linked to crustose coralline algae with distinct chemical and microbial signatures. Sci Rep *11*, 14610. 10.1038/s41598-021-94096-6.