Modern natural products drug discovery and its relevance to biodiversity conservation

C. Benjamin Naman, Christopher A. Leber, and William H. Gerwick*

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, San Diego, California 92037, United States

*Author for correspondence: email wgerwick@ucsd.edu; Phone 858-534-0578

C.A. Leber: cleber@ucsd.edu

C.B. Naman: bnaman@ucsd.edu

Running Title: Natural Products Biodiversity Conservation

Summary

The small molecules with adaptive functions made by plants, animals and microbial life forms are known as natural products, and have formed the basis of much of our current pharmacopeia. Up to 70% of all drugs on the market today have an origin or inspiration from Nature. As a consequence of the incredible importance of these natural products, both in terms of their life saving impacts as well as monetary value, the life forms that produce these materials also have direct societal worth. Realizing the value of biodiversity has provided new arguments in favor of preservation and conservation efforts, and allows for tangible evidence that the general public both appreciates and approves. This chapter provides a description of these arguments as well as two case examples; one from the island of Coiba in Pacific Panama, and another from the Caribbean island of Curaçao.

Keywords: Natural products, Drug discovery, Biodiversity, Conservation, Panama, Coiba, Curaçao, Cyanobacteria, Coibamide, Curacin A

Natural products (NP) drug discovery research is inherently and intricately intertwined with biodiversity conservation. NP research relies explicitly on the availability of biodiversity as the source material from which new drugs, pharmacological probes, and interesting chemical leads may be obtained. The fundamental goal of NP drug discovery is to isolate and determine the structures of small molecule NP chemicals, frequently referred to as secondary metabolites, and to aid in their development as useful societal materials.

As a general principle, different life forms tend to elaborate different types of NPs. For example, the NPs of terrestrial and marine microorganisms, fungi, invertebrates, and higher plants each possess distinctive and unique structural features. This results from the production of NPs, via enzymatic reactions, catalyzed by proteins programmed by DNA sequences that differ greatly between the source organisms. These differences in DNA reflect the disparate evolutionary forces acting on these organisms. Furthermore, geographically diverse samples tend to also be chemically diverse, and thus, the examination of the same or similar organisms from different locations has been a productive approach to the discovery of novel chemical entities. As NP bioprospecting has become worldwide in scope, this has necessarily given rise to collaborative interactions between scientists in different countries as well as government involvement in the regulation of international science.

There have been many benefits to conducting NP drug discovery research on a worldwide scope; however, this has necessitated international policies and protocols so as to prevent potential harm and other problems. For example, harmful microorganisms, insects or other invasive species might be transported along with scientific samples, and cause significant environmental damage. In another sense, international agreements such as the Convention on Biodiversity Preservation have been enacted along with subsequent accords (Cartagena in 2003 and Nagoya in 2014) to ensure that the proper Memorandum of Understanding and benefit sharing agreements are in place, in the event that an economically valuable compound be discovered

or developed from indigenous life forms, or “genetic resources”, of a foreign country. Microorganisms, which are especially easily transported, have been the focus of many industrial drug discovery research programs due in large part to the scalability of their growth and compound production. These latter aspects of NP drug discovery are addressed in another chapter of this book and are not further discussed here.

Studies of the biosynthesis of NPs in microorganisms over the last two decades have revealed that the genes encoding the biosynthetic enzymes tend to be clustered, and this has greatly accelerated their discovery, characterization, and downstream production by genetic methods. This has also enabled genomics-based NP drug discovery once the DNA sequence of an organism has been determined. DNA sequence information is not only a helpful tool for NP drug discovery, but also for the taxonomic description of biodiversity itself. Development of a genetic basis for determining the taxonomy of a given organism, such as by the 16S rRNA gene sequence for prokaryotes, has greatly improved our ability to accurately identify NP producing organisms, as well as appreciate that there is much greater diversity in microbial life forms than was previously thought. Ultimately, this information is a critical part of the data portfolio needed to better understand the value and the benefits of a biodiverse planet.

Natural Product Drug Discovery

Historically, NP drug discovery began with research on traditional medicines and naturally produced toxins. Morphine is a well-known example of a naturally produced substance with a long history of utilization in its crude form. In the early 1800s, it was subsequently purified and utilized for medicinal purposes as a pure substance. This discovery spurred great interest into the chemical composition of plant pharmaceuticals, and this interest later expanded to include fungi, microorganisms, and most recently, marine life forms. In their purified forms and not accounting for existing systems of traditional medicine, unmodified NPs account for approximately 5% of the drugs currently approved worldwide. Furthermore, approximately another 50% of

such approved drug agents represent chemicals that were either synthetically modified from a NP or designed with inspiration from the knowledge of a NP's molecular structure. These modifications have typically been made to improve beneficial drug properties for the final product, such as increased efficacy, reduced toxicity, more desirable routes of delivery, or for production cost effectiveness. Sometimes, the structure of the final drug product has been changed so significantly from the original lead molecule that it is difficult to discern their relationship.

Some early success stories from NP drug discovery have entered the common vernacular. For example, the alkaloidal substance quinine, which is added to "tonic waters" worldwide and is responsible for the bitter taste, was first administered to prophylactically combat malaria. More recent discoveries, made possible by technological developments that have allowed for the bioprospecting of microbial organisms, have significantly contributed to increasing the average human lifespan on a global level. In particular, the "golden age of antibiotics drug discovery" during the 1940s to 1960s included the cephalosporin, penicillin, tetracycline and vancomycin antibiotics. A current strategy for drug discovery and delivery involves the linking of highly efficacious NP drugs and drug candidates to designer antibodies, forming antibody-drug conjugates (ADCs), which enable highly specific drug targeting, increased efficacy and reduced toxicity for patients. For example, the anticancer agents, trastuzumab emtansine and brentuximab vedotin are ADCs that utilize derivatives of terrestrial and marine microbial NPs as "warhead" toxins to enact their therapeutic effects. Emtansine is a simple modification of the NP toxin maytansine whereas brentuximab vedotin possesses a derivative of the cyanobacterial metabolite dolastatin 10 (figure 1). Still, unmodified NPs such as ecteinascidin (ET-743 or trabectedin) and homoharringtonine, and various NP chemical derivatives such as the halichondrin A analogue, eribulin, also continue to be approved as new molecular entities for use in the clinic (figure 2).

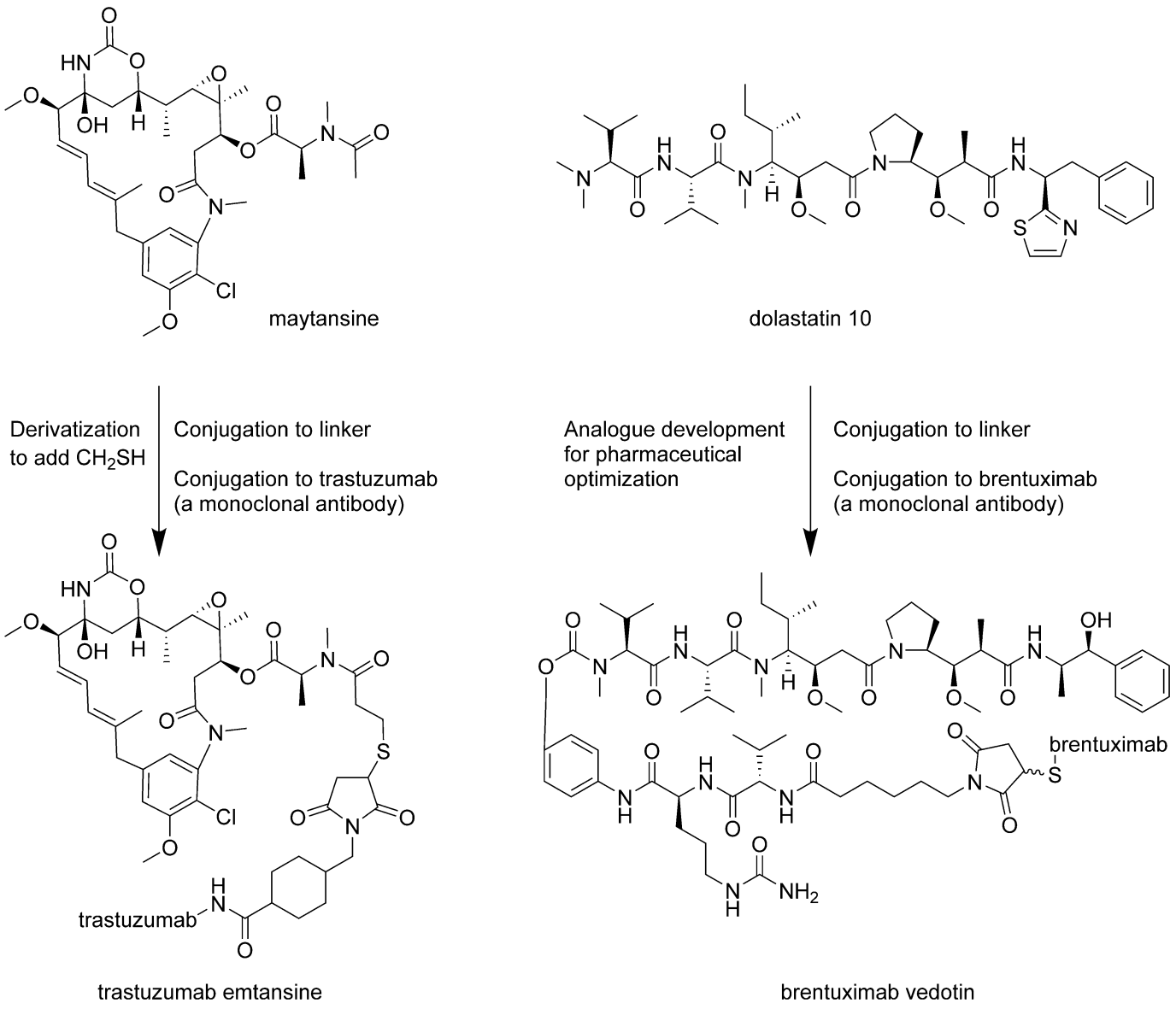

Figure 1. Chemical structure of the NP maytansine and antibody-drug conjugate trastuzumab emtansine along with the NP dolastatin 10 and antibody-drug conjugate brentuximab vedotin.

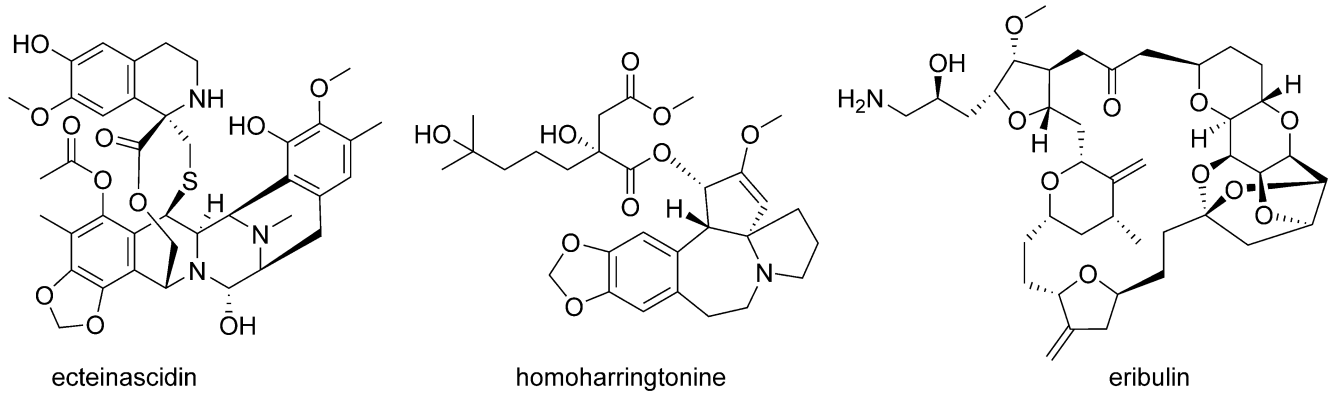

Figure 2. Chemical structures of the NP drugs ecteinascidin and homoharringtonine, and NP derivative drug eribulin.

The advent of the Self-Contained Underwater Breathing Apparatus (SCUBA) enabled chemists, beginning in the 1960s, to begin to investigate the unique chemical constituents of marine organisms. Notable early successes include the isolation of prostaglandins in gram quantities from the Caribbean Sea Whip, *Plexaura homomalla*, characterization of the exceptionally potent seafood toxin saxitoxin, and discovery that marine algae of the phylum Rhodophyta produce an abundance of halogenated terpenoid and polyketide NPs. As modern technologies and tools have improved over the years, investigations of marine life have risen to the forefront of NP research. Many different and innovative approaches have emerged, including sourcing marine organisms from new and unique niches, conducting ecology-driven investigations, developing novel laboratory culture conditions, and utilizing genomic approaches. Cyanobacteria have been of particular interest for drug discovery due to their abundant biosynthetic capacities to elaborate structurally diverse NPs with potential biomedical application.

This has been particularly true in the search for new cancer chemotherapeutic agents. For example, the potential anticancer depsipeptide 'coibamide A' was purified from a Panamanian cyanobacterium collected using SCUBA from a reef pinnacle in the Coiba National Park. Coibamide A is a potent cancer cell cytotoxin and represents an exciting lead molecule, as described later in this chapter. Another potential anticancer lead

molecule is the alkaloid curacin A, which was isolated from a sample of *Moorea producens* (formerly *Lyngbya majuscula*) collected in Curaçao. Curacin A is a potent cytotoxic agent, and its discovery sparked significant conservation efforts in the island country for more than a decade. Many more examples of biologically active cyanobacterial NPs have been reported, such as apratoxin A and largazole, and there are several ongoing research programs actively exploring their potential development. The structures of these molecules are presented in figure 3.

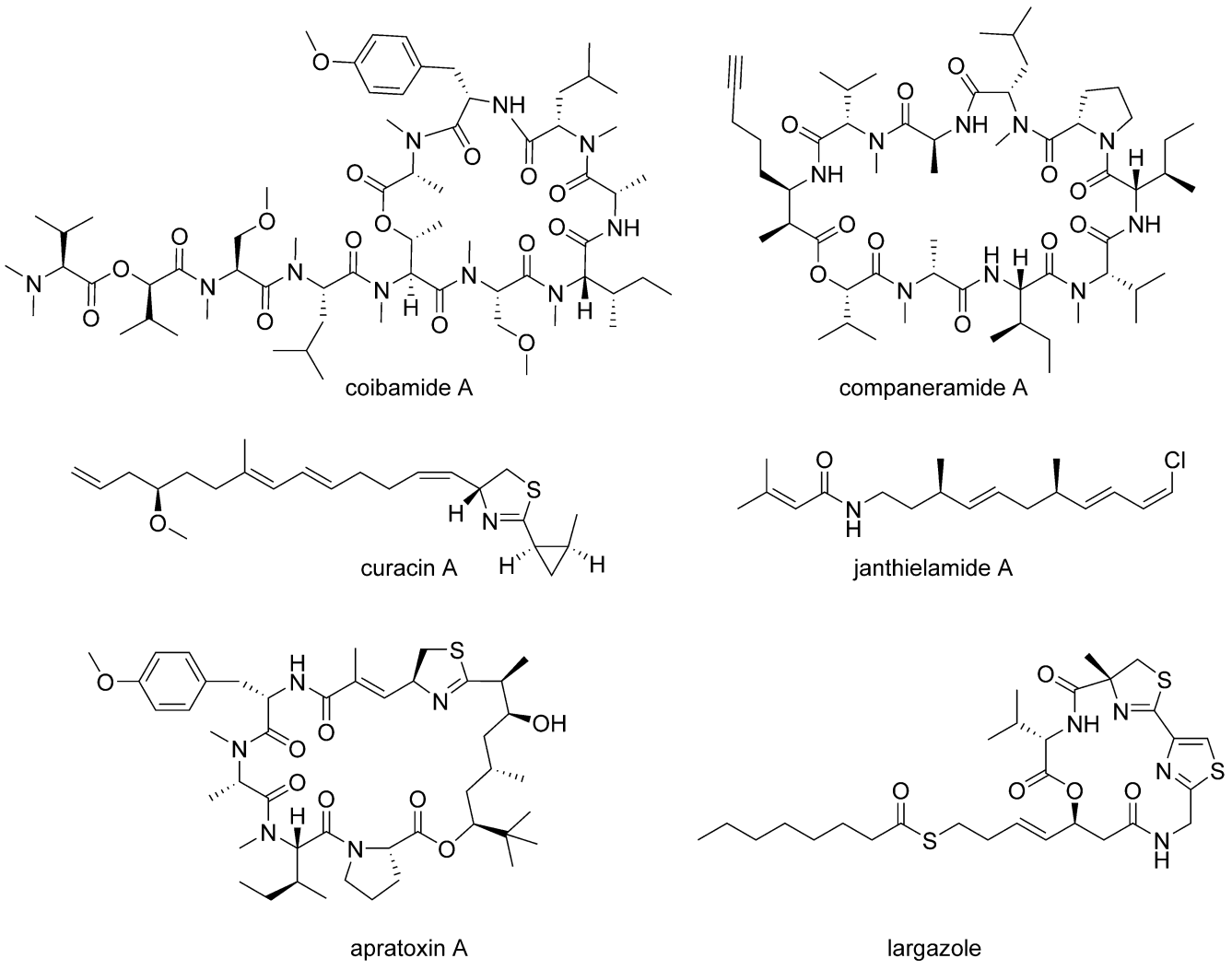

Figure 3. Chemical structures of the biologically active cyanobacterial NPs coibamide A, companeramide A, curacin A, janthielamide A, apratoxin A, and largazole.

Policy Regarding Bioprospecting

Several sets of international conventions and policy agreements are in current enforcement that relate to the preservation and appropriate usage of biodiversity. An early example is the Convention on the International Trade of Endangered Species of Wild Flora and Fauna (CITES). Devised by the International Union for the Conservation of Nature (IUCN) in the 1960s and enacted in the early 1970s, CITES put in place regulations on the trade of endangered species to prevent their extinction and to slow the rate of biodiversity decline worldwide. The list of protected plant and animal species is maintained and periodically updated by IUCN, and also applies to products made from those organisms. Furthermore, participating countries have the opportunity to request protection of indigenous wildlife to prevent its unauthorized collection and distribution to other nations. Although CITES does not currently regulate the trade of microbial organisms, it has been successful in slowing the rate of eukaryotic species extinction, and has served as an important mechanism by which to raise societal awareness of biodiversity and its importance.

The more recent Convention on Biological Diversity (CBD) was presented at the 1992 United Nations Conference on Environment and Development. This conference was held in Rio de Janeiro at the so-called “Rio Earth Summit”; the convention itself has often been referred to as the “Rio Convention”. Several issues relevant to bioprospecting were raised by CBD, such as the "need to share costs and benefits between developed and developing countries" along with "ways and means to support innovation by local people". Prior informed consent is required in order for foreign investigators to gain access to the sovereign genetic resources of a state. In practice, this has led to intellectual property agreements that ensure the equitable sharing of profits, and are negotiated in advance of bioprospecting research programs. The CBD has become the most widespread policy adopted internationally that relates to biodiversity and bioprospecting. Unfortunately, in a few cases an unintended consequence of the CBD has been the creation of unreasonable

expectations for the monetary rewards of bioprospecting, thereby inhibiting research on the NPs of organisms from those countries. In fact, the rate at which newly discovered NP molecules gain regulatory approval and clinical use is notoriously low, and this process is coupled to an immense financial outlay. The different outcomes expected by some developed and developing countries during advanced negotiations has been cited in the past as a reason that strategic partnerships fail to be formed with higher frequency. The United States signed but never ratified the CBD, effectively meaning that federally funded researchers are required to uphold the policies of the CBD whereas private industries are not. Nevertheless, many productive and mutually beneficial partnerships have been created between developed and developing countries in NP drug discovery research, and examples of two of these will be presented later in this chapter.

A supplementary agreement was added to the CBD in 2010, called the "Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization" ("Nagoya Protocol" or ABS). The terms of the ABS did not go into effect until late 2014, after it had been signed and ratified by 50 member countries or "parties". Some of the major issues addressed by the ABS were the lack of requirement for transparency of research progress, the need to raise awareness of the importance of biodiversity in the countries both sharing and accessing genetic resources, as well as the necessity for the provision of a clear and consistent framework for benefit-sharing and prior informed consent. Some members of the scientific community have voiced concerns that the rigidity of the ABS guidelines could hamper conservation and research efforts. One such criticism of this agreement is that it requires the establishment of national focal points and competent national authorities through which all access and benefit-sharing agreements are handled. The intent of this particular article of ABS appears to be prevention of back-room dealings amongst individuals wishing to circumvent benefit sharing, as well as to unify the procedures for accessing biodiversity in countries both large and small. However, the fear and likely reality is that this requirement will actually lead to further delays in relationship building, as well as overwhelm national focal point offices in biodiversity hotspots. Through the end of 2015, the United States had not

signed or ratified the new ABS agreement. The long-term effect of implementing these new ABS articles is not yet clear. The development of benefit-sharing agreements has always been a long term and committed process, and not enough time has passed to yet know these consequences. However, the Nagoya Protocol Implementation Fund, a sizeable trust that offers grant money to research programs with partnerships in the private sector, has begun to provide sizeable research funding to encourage ratification and implementation of the Nagoya Protocol.

Biodiversity Estimates

Biodiversity can be practically defined in a number of different ways: number of species or species richness, relative species abundance or evenness, concentration of endemic or rare species, as well as considerations of alternative taxonomic levels or focus on particular taxonomic clades. Each measure of biodiversity has its own insights and shortcomings, but a universally shared limitation in quantifying biodiversity is the uncertainty associated with our knowledge of what is actually present. Our planet is host to tremendous amounts of biodiversity, but the great majority of this has not yet been studied or even discovered. There are accepted taxonomic names and descriptions for about 300,000 land plants and approximately 1.9 million species of animals. In comparison, the total number of plant species on Earth is estimated to be greater than 450,000, and the number of insect species alone is thought to range between 5 and 6 million! Estimates for total eukaryote diversity, as suggested by various models, range from 2 to 100 million, with some even suggesting that no estimate is realistic given our incomplete state of knowledge, especially in regards to fungal and insect diversity.

We have even less knowledge of prokaryote biodiversity, though the initiation of metagenomic research and the development of next-generation sequencing technology have greatly expanded our appreciation of the magnitude and ecological importance of the diverse global microbiome. Late in the 20th century, when the

study of bacteria relied heavily on traditional culture methods, bacteria were classified into 11 main phyla. As of 2004, after approximately 20 years of metagenomics research, the number of detected bacterial phyla had risen to 53. The advent of next-generation sequencing has expanded our understanding of prokaryotic diversity even further. As of 2014, over 4 million 16S rRNA gene sequences have been deposited in publicly available databases, and sequence data-based rarefaction curves estimate the number of prokaryote phyla to range from about 1,100 to about 1,350. Remarkably, estimates of species diversity in prokaryotes extend from approximately 30,000 to some eight orders of magnitude greater (e.g. on the order of a trillion); some even question the appropriateness of the 'species concept' when applied to prokaryotic life forms.

The continued development in understanding prokaryotic genetic diversity has been closely accompanied by increases in knowledge of the diversity of prokaryote ecological function. Prokaryotes are ubiquitous throughout Earth's ecosystems, and they serve myriad ecological roles of tremendous importance. These roles include primary production via photosynthesis and chemosynthesis, nitrogen fixation, methanogenesis and methane oxidation, nutrient remineralization, and initiation of countless other oxidation and reduction reactions that transform and significantly impact the environments in which eukaryotes (and other prokaryotes) live.

Eukaryote biodiversity is not uniformly distributed across the surface of the Earth, but is instead geographically concentrated. Abundant species tend to inhabit larger geographic ranges, whereas less abundant species tend to be more geographically concentrated. These more rare species of limited abundance and geographic range (= endemic species) often co-occur with other rare species so as to create areas of high biodiversity known as biological 'hotspots'. Just as eukaryotes have biogeographic patterns, there is pronounced spatial and temporal variability in microbial communities. Heterogeneous environments present diverse microhabitats and niches that support distinct and specific microbiomes. For example, the microbial community that inhabits a particular swath of oxic ocean sediment will greatly differ from the microbial

communities in the suboxic and anoxic sediment layers below, and the ocean water above. Similarly, microbial communities differ greatly between such niches as sponges, corals, algal surfaces, fish intestines and wood boring worms. Considering the number of such microhabitats, each with its specific microbiome, makes more reasonable the estimates of the enormous number of prokaryotic life forms.

The vast biodiversity that is hosted by our planet, especially the microbial diversity, holds great promise for the continued productivity and success of NP research. The variety of prokaryotic ecological functions combined with the diversity of environments they inhabit provides some indication of the likely extent of metabolic capabilities that these organisms possess. For this reason, each newly investigated species has significant potential to yield biologically useful NPs.

Threats to Biodiversity

There is considerable interest to describe Earth's complete biodiversity in advance of any large-scale extinction events. Anthropogenic impacts including, most notably, habitat destruction and over-hunting and fishing have been particularly efficient in eroding biodiversity. It has been estimated that the current extinction rate for eukaryotes is at least 1000 times greater than the background extinction rate. There is grave concern that global climate change will exacerbate this situation with resulting non-linear increases in extinction rates.

Loss of biodiversity is of great concern to ecologists and NP researchers alike in that each extinction of a species represents an irretrievable loss to the planet as well as to society. With each eukaryotic extinction, a piece of an ecosystem is lost, permanently altering how that ecosystem is structured and how it functions. Coupled with each eukaryotic extinction is the destruction of any number of microbiomes that exist within that organism, or otherwise rely on it for continued existence (e.g. coprophilous fungal microbiomes). NP

researchers are thus implicit stakeholders in the preservation of biodiversity, making partnerships between NP drug discovery and biodiversity conservation both logical and necessary.

Classification of Biodiversity Resulting from Bioprospecting; Cyanobacteria as an Example

The phylum Cyanobacteria represents a broad genetic group; until recently, proper classification systems were insufficient to properly delineate phylogenetic relationships in this group. Bioprospecting efforts have significantly contributed to developing a better understanding of the relationships within this group. Previously, morphology and culture condition characteristics were used to define genera and species of cyanobacteria; however, 16S rRNA gene sequencing as well as Multi-Locus Sequence Typing have revealed much more genetic diversity in the cyanobacteria, and completely altered our understanding of their evolutionary relationships. For example, the genus *Lyngbya* was previously thought to be responsible for over 40% of the total number of NPs produced by cyanobacteria. It was not until specimens of this genus were examined phylogenetically that *Lyngbya* was revealed to be polyphyletic. Efforts to correct this mis-classification resulted in the formation of a new genus, *Moorea*. It is interesting to note that when the 16S rRNA gene sequences were evaluated using the Bayesian method, these morphologically similar groups formed distinct and distant clades (figure 4).

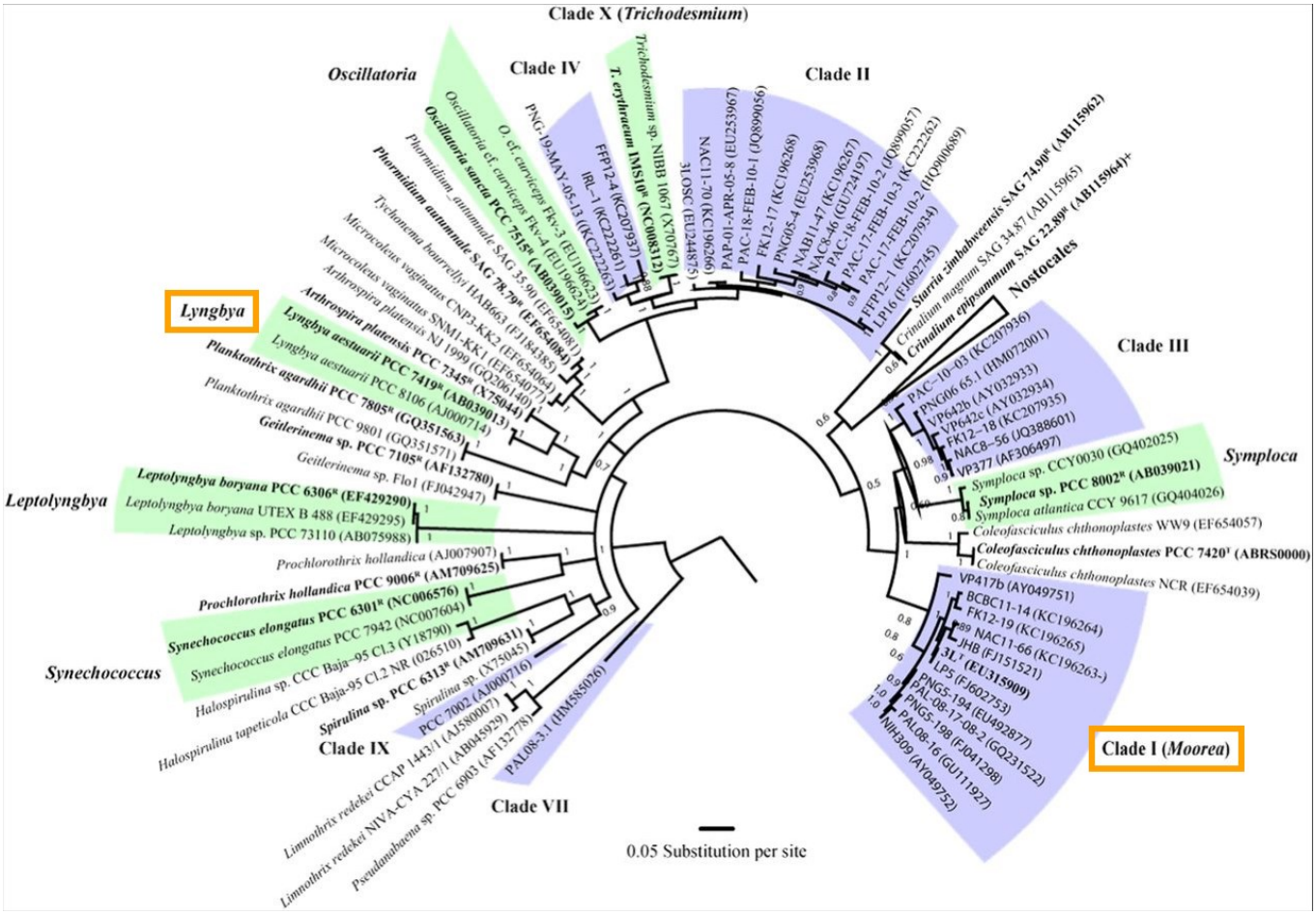

Figure 4. Evolutionary tree of marine cyanobacteria known to produce NPs based on 16S rRNA gene sequences using the Bayesian method. [Reproduced, with permission, from Engene N, Gunasekera SP, Gerwick WH, Paul VJ. Phylogenetic inferences reveal a large extent of novel biodiversity in chemically rich tropical marine cyanobacteria. *Appl. Environ. Microbiol.* 2013;**79**(6):1882–88.] NAC11-66 corresponds to *Moorea producens* (formerly *Lyngbya majuscula*). The genera *Lyngbya* and *Moorea* are outlined in red boxes.

In addition to the reclassification of previously described taxa, phylogenetic investigations can also lead to more accurate initial descriptions of newly isolated taxa. Due to systemic structural issues in the funding of scientific research, it is common that phylogenetic studies are only conducted on organisms once their potential value from bioprospecting research has been established. Consequently, deeper insights into the extent of cyanobacterial biodiversity as well as their capacity for NP biosynthesis have been hindered.

For example, a cf. *Symploca* sp. collected in Curaçao yielded a new NP, janthielamide A (figure 3). Initial taxonomic assignment was based solely on morphological characteristics; however, examination of its 16S rRNA gene sequence suggested that it belonged to an evolutionarily distant and undescribed genus. Similarly, a Panamanian cyanobacterium that yielded the antiplasmodial NP companeramide A (figure 3) was initially described as a *Leptolyngbya* sp. However, phylogenetic analysis indicated that it was more similar to, but significantly distinct from, the genus *Symploca*; a new genus, *Hyalidium*, has been proposed for this organism. Another Panamanian example comes from the compound coibacin A, a selective antileishmanial and anti-inflammatory agent produced by a cyanobacterium thought to be of the genus *Oscillatoria*, based on its morphology. However, phylogenetic studies revealed that it is distinct and likely a representative of a cyanobacterial genus that is currently undescribed. Thus, it is unclear how many species of cyanobacteria have been misidentified in the past and have yet to be reclassified. Nonetheless, it is clear that bioprospecting and biodiversity studies are contributing significantly to these efforts.

Biodiversity Partnerships, Bioprospecting and Conservation Efforts

Much of Earth's biodiversity is concentrated in tropical terrestrial and shallow water sub-tidal environments. These same geographical locations are comprised of mainly economically-disadvantaged countries that struggle to meet the basic infrastructural, medical and educational needs of their citizens. As a result, it can be difficult to allocate resources for the preservation of biodiversity because this heritage is not fully appreciated or necessarily prioritized. However, by identifying and developing the economic value of biodiversity in specific and concrete terms, the goals of economic development and biodiversity preservation can become synergistic. Such has been the case in Panama as a result of a two decade long effort that involved an in-country renaissance of support for the natural sciences that synergized with an NIH-Fogarty International Center-sponsored project, namely the Panama International Cooperative Biodiversity Group (Panama ICBG).

The overall ICBG program was established in 1992 as a cooperative effort between the U.S. National Institutes of Health, National Science Foundation, and Agency for International Development, and has been coordinated by the Fogarty International Center. The aim of this program has been to foster incentives for biodiversity conservation and sustainable economic growth through scientific training and research, particularly focused on NP drug discovery. Participants in the ICBG programs have included not only the U.S. agencies and university researchers, but also the government agencies and research centers in the partner countries, and a great exchange of scientific capability and training has accompanied the benefit sharing agreements of these efforts. The first ICBG programs were initiated in partnership with Cameroon and Nigeria, Costa Rica, Peru, Suriname, and cooperatively between Argentina, Chile, and Mexico. Several additional ICBG programs were subsequently organized, with the Panama ICBG among them, and a few are ongoing with funding secured through at least 2019.

The diverse experiences of the extended ICBG effort in Panama, which had the central focus of discovering novel therapeutic agents from tropical marine and terrestrial life forms, especially against neglected parasitic diseases and cancer, resulted in a number of discoveries which have had a positive impact on the country's preservation of biodiversity. In hindsight, it was the remarkable insight and inspiration of the ICBG program itself that sowed the seeds for much of this success. The ICBG programs were designed to intimately link the exploration of biodiversity for potentially useful products along with its characterization and preservation. Additional goals were to enhance economic development, training, and infrastructure development in the host country. The embodiment of this within the Panama ICBG was to locate much of the actual NP drug discovery research and conservation efforts in Panama, thereby ensuring the training of Panamanian students and faculty, development of scientific infrastructure, and greatly increasing the country's appreciation of the value of its biodiversity.

To a significant degree, the ability to conduct substantial research activities within Panama was made possible as a result of the purchase and placement of several key pieces of scientific instrumentation in the host country. For example, the NCI, the Fogarty International Center and the Smithsonian Tropical Research Institute collectively purchased for the Panama ICBG effort a research grade 300 MHz NMR instrument. This was placed in the Smithsonian Tropical Research Institute's Tupper Campus in Panama City, and enabled high quality NP research to occur in-country. Subsequently, a second high field instrument, this time operating at 400 MHz, was purchased with Panamanian government funds and placed at their nascent City of Knowledge research facilities. These were paradigm-shifting events with wide ranging consequences. Panamanians receiving graduate and postdoctoral education in NP and organic chemistry elsewhere in the world could now return to Panama and practice their disciplines to the level that they were trained, making their return home a more viable and attractive career path. Upon their return, they could not only conduct world-class NP science, but also train and develop the next generation of such scientists within their own nation state and its academic programs. This has had the consequence of opening up academic science to an increasingly large cadre of young and well-trained Panamanian chemists, growing the in-country capacity and appreciation for science and its aspirations.

A major geographic focus of efforts in Panama centered on the island of Coiba, a roughly 200 square mile island some 25 miles off the West Coast of the country. From 1919 to 2003, Coiba served as home to a notorious penal colony, the fearsome nature of which had the unintended consequence of keeping the majority of the island free from habitation or resource utilization. When the prison closed, it was thus prime real estate for potential development. At about this time, the Panama ICBG project began explorations of the island's exceptionally rich terrestrial and marine flora in search of unique NPs and with a special interest in compounds active in antiparasitic and anticancer screens. One organism of interest, collected from several sub-tidal environments around the island and appearing as gelatinous red strands, was identified as a cyanobacterium *Leptolyngbya* species. Exceptionally potent cancer cell toxicity was noted in the crude

extract, and this was ultimately traced to a novel cyclic depsipeptide, given the trivial name “coibamide A”. Coibamide A became a very high priority anticancer lead compound as a result of its novel chemical structure, very potent cancer cell toxicity, and unique though incompletely characterized mechanism of action. Total chemical synthesis of coibamide A has been hampered by its propensity for epimerization, decomposition, and conformational flexibility. However, recent mechanistic studies have revealed that it induces cancer cell death via mTOR independent autophagy, and its specific molecular target is the Sec61 protein, an endoplasmic reticulum membrane protein translocator.

At about the same time as the discovery of coibamide and several other notable NPs from Coiba’s unique flora and fauna, it was becoming clear that its biodiversity required protection. If steps were not taken to protect the island, it would have fallen prey to commercial interests and been developed as a tourist destination with resort hotels and their associated facilities. Several key individuals from the Panama ICBG program became deeply invested in the preservation of Coiba, namely Drs. Todd Capson and Alicia Ibanez. As a result of their efforts, along with many others in non-governmental organizations and the Panamanian National Government, Coiba Island was named a World Heritage Site by UNESCO in 2005. Management of the park, especially in regards to the prevention of illegal activities such as commercial fishing and timber harvests, has been difficult to oversee, but with steady progress being made. The Coiba National Park thus stands as one of a number of lasting testaments and accomplishments of the Panama ICBG program, and part of its natural and biodiverse beauty can be appreciated in figure 5.

Figure 5. Photographs of the natural beauty and biodiversity of Coiba National Park in the Gulf of Chiriqui on the west side of Panama. [Reproduced with permission from the copyright holder, Alicia Ibáñez].

Another example of natural product driven conservation efforts derives from our previous work in the Southern Caribbean island of Curaçao. This low lying arid island has played an important role as an access point between much of South America, especially Venezuela, and the rest of the world. Early on, its exceptional deep-water port made it a practical site for trade and commerce. With the discovery of oil in Venezuela's nearby Maracaibo Basin, Curaçao became the logical location for a major oil refinery, which has now been in operation for roughly 100 years. The storage and refinement of oil on Curaçao made it a location of considerable strategic importance during the Second World War. Moreover, an exceptional mountainous deposit of calcium carbonate exists in the southern portion of the island, and this has been mined and shipped around the world for many decades. Whereas these industries have certainly contributed to the economy of the island country, they have also left their indelible anthropogenic mark on the island's once natural landscape. Furthermore, the vegetation of Curaçao has been enormously damaged by the free-range pasture of goats, resulting in a desert-like environment populated by cacti, thorny bushes and other goat tolerant vegetation. Curaçao is thus a curious mixture of the relatively pristine and the environmentally degraded; these have existed side-by-side for many years. However, a slow but steady economic resurgence is evident, with hotels, private villas, and destination resorts being introduced to the island. This has initiated a new round of environmental pressure on the island, as bay-front and beachside touristic facilities and housing have recently appeared. One such conflict arose in the Barbara Beach region of the Spanish Bay on Curaçao's Southwest coast.

In 1993, our laboratory initiated collections of marine algae and cyanobacteria from the island of Curaçao. Hosted through the increasingly sophisticated marine laboratory of CARMABI (Caribbean Research and Management of Biodiversity), we made collections of shallow water cyanobacteria from various locations along the leeward side of the island, from Playa Kalki in the Northwest to Punt Kanon in the Southeast. One such collection was made of trellises of red filaments descending from mangrove roots into the shallow

waters of Spanish Bay. These trellises were comprised of uni-cyanobacterial growths, some as much as 40 cm long, that accumulated into soft tufts easily collected by hand. This cyanobacterium is now known to be *Moorea producens*, with the common name of "Mermaid's Hair". Co-existing in this unique habitat were stinging hydrozoans, scorpion fish, *Cassiopeia*, the occasional young barracuda and a diversity of other juvenile fish species. At the time of first collection, this region of Spanish Bay was only reachable by a nearly 30 minute swim from the closest access at Barbara Beach, and lacked any significant point of interest to anyone other than marine scientists.

Subsequently, a cell toxicity assay run as part of a screen for new antiviral agents at the then Syntex Corporation in Palo Alto revealed the extract of these trellises to possess phenomenal levels of cancer cell cytotoxicity. We proceeded to isolate the active compound, named "curacin A", and determine its planar structure, which was first published in 1993 in the *Journal of Organic Chemistry*. Ensuing mechanistic studies revealed curacin A to be an exquisitely potent cytotoxin that acted at the colchicine site of microtubules, inhibiting their polymerization and thereby disrupting the ordered separation of chromosomes during mitosis. With determination of the absolute stereoconfiguration of curacin A by a combination of chemical degradation and synthetic chemistry efforts, along with the development of total synthetic routes to the molecule, its status as an anticancer lead molecule was established in the field.

Advancement of curacin A to this status had a number of consequences, including that aspects of its biology, ecology, and biosynthesis became of great interest to the scientific community. It also helped to accentuate the point that this forgotten bay in Curaçao, with its fringe of mangroves and tufts of cyanobacteria, was a habitat with a specific and very tangible value to society. The average person broadly appreciates the need for new, more efficacious medicines to treat diseases such as cancer. So through seminars given to the general public in Willemstad, television interviews and newspaper stories, and other communications

provided by the CARMABI research station in Curaçao, a greater understanding and appreciation of the value of the local unique marine biodiversity spread throughout the island's populace.

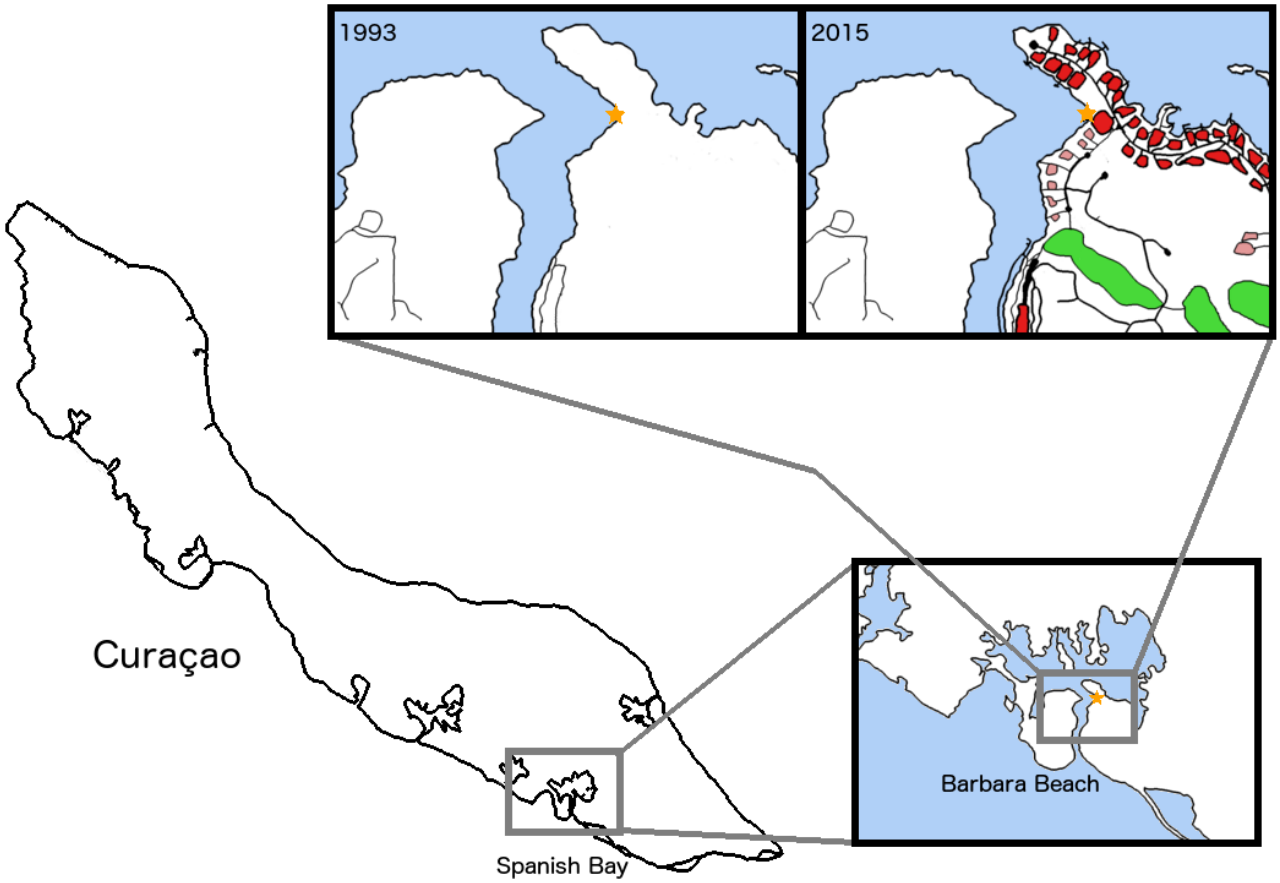

Figure 6. Outline of the island of Curaçao with expansions of the Barbara Beach region and Spanish Bay where the curacin A producer, *Moorea producens*, was collected in 1993. Yellow star denotes the specific site of the mangrove from where *M. producens* was collected. Red blocks represent housing developments, green patches signify the Santa Barbara golf course, and pink blocks are prospective development sites.

In the late 1990s, interest in commercial development of the Barbara Beach area grew. Plans were laid for a resort-style hotel, as well as an array of upscale residences, complete with individual boat dock facilities and swimming pools. These plans involved radical alteration of the waterfront vegetation in a portion of Spanish Bay, and possibly included the complete removal of the narrow fringe of mangroves that had provided the unique habitat from which the Mermaid's Hair was collected. Legislation was introduced in Curaçao to

block this development, and this was successfully upheld for a number of years. The argument most easily understood and appreciated in this situation was that the natural resource, specifically *M. producens*, potentially held a real and tangible value to society, and so was worthy of preservation. However, after a number of years in the absence of realization of this value in monetary or material terms, the arguments became less convincing, and so the developers were ultimately successful in overcoming these environmental impact concerns (Figure 6). Today, a luxury hotel, fringing docks, speedboats, a golf course and villa-style homes occupy this area, which was previously only the domain of cacti, lizards, mangroves, and Mermaid's Hair.

Summary and Future Outlooks

The efficacy of NP research in discovering compounds worthy of pharmaceutical utilization is supported by a history of successes and the current importance of NPs and associated derivatives in drug markets worldwide. The profound impact of discoveries in this field has been well-recognized, including the joint awarding of the 2015 Nobel Prize in physiology or medicine to the two lead researchers responsible for the late 20th century discovery of the naturally occurring antiparasitic drugs avermectin and artemisinin. Paired with the preponderance of Earth's biodiversity that is yet to be explored, the field of NP research has tremendous potential for continued advancement, as well as further improvements to global human health through the provision of new more efficacious pharmaceutical agents. International policies such as CBD, the Cartagena and Nagoya accords, and even yet unimagined agreements are important in protecting the rights of developing nations as well as encouraging their interest in biodiversity exploration and conservation efforts. These policies also serve to facilitate related worldwide research efforts and scientific training programs, ultimately benefitting society as a whole by many direct and indirect avenues. However, as global climate change begins to compound the already burgeoning anthropogenic threats to biodiversity, the available opportunities for discovery leading to sustainable economic growth and usage of biodiversity is

progressively lessened. Faced with this grim reality, it is absolutely imperative that conservationists and NP researchers unite in their efforts to protect and study biologically diverse habitats. With adequate funding, proper support from policy makers and governments worldwide, and the formation of international partnerships, significant efforts can and must be made to preserve our planet's incredible and invaluable biodiversity.

Suggested Further Reading

Calteau A, Fewer DP, Latifi A, Coursin T, Laurent T, Jokela J, et al. Phylum-wide comparative genomics unravel the diversity of secondary metabolism in cyanobacteria. *BMC Genomics* 2014;**15**:977.

Cragg GM, Katz F, Newman DJ, Rosenthal J. The impact of the United Nations Convention on Biological Diversity on natural products research. *Nat Prod Rep* 2012;**29**:1407–23.

Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. *Nat Rev Drug Discov* 2015;**14**(2):111–29.

Kingston DGI. Modern natural products drug discovery and its relevance to biodiversity conservation. *J Nat Prod* 2011;**74**(3):496–511.

Kursar TA, Caballero-George CC, Capson TL, Cubilla-Rios L, Gerwick WH, Gupta MP, et al. Securing economic benefits and promoting conservation through bioprospecting. *Bioscience* 2006;**56**(12):1005–12.

McCarthy DP, Donald PF, Scharlemann JPW, Buchanan GM, Balmford A, Green JMH, et al. Financial costs of meeting global biodiversity conservation targets: Current spending and unmet needs. *Science* 2012;**338**(6109):946–9.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. *Nature* 2000;**403**:853–8.

Pimm SL, Jenkins CN, Abell R, Brooks TM, Gittleman JL, Joppa LN, et al. The biodiversity of species and their rates of extinction, distribution, and protection. *Science* 2014;**344**(6187):1246752-1—10.

Tan G, Gyllenhaal C, Soejarto DD. Biodiversity as a source of anticancer drugs. *Curr Drug Targets* 2006;**7**(3):265–77.

Uemura D. Exploratory research on bioactive natural products with a focus on biological phenomena. *Proc Jpn Acad, Ser B* 2010;**86**(3):190–201.