A Thesis Presented to

The Faculty of Alfred University

Analysis of *Journal of Natural Products* Publications for Novelty in Structures

by

Madison Muehl

In partial fulfillment of
the requirements for
the Alfred University Honors Program

5/10/19

Under the Supervision of:
Chair: Dr. John D'Angelo, Associate Professor of Chemistry
Committee Members:
Dr. Geoffrey Lippa, Assistant Professor of Biology
Dr. Heather Zimbler-DeLorenzo, Associate Professor of Biology

Table of Contents

Research Statement

Many of the medicines that we consume have come from the isolation of chemical compounds from natural sources. These natural sources include plants, fungi, bacteria, and even some animals like marine sponges and nematodes. These natural products have been used for herbal medicine for centuries.

Once scientists believe that they have identified a natural source that could potentially yield useful natural products, they extract the natural product from the source and test for its bioactivity. The bioactivity of a compound is how the compound affects the natural sources biological system. If researchers understand the bioactivity of a compound, they might begin to understand how this compound can be developed into a drug.

Isolating natural products that have the potential to become new therapeutics is both difficult and time consuming. Researchers are constantly under pressure to fight against the ticking clock of public health issues such as antibiotic resistance. As bacteria become resistant to antibiotics, researchers are searching for new compounds that may have antibiotic bioactivity. If new antibiotic compounds can be identified, our chances of being able to combat antibiotic resistance increase by targeting the resistant bacteria in a different way. Researchers refer to these different ways for drugs to combat infections as “mechanisms of action”.

Discovering new mechanisms of action may be difficult if researchers are returning to the same types of natural sources over and over. Currently, over 70,000 vascular plants have been surveyed for novel therapeutic compounds. Since so much

effort has been put into compound extractions from vascular plants, it is important to understand where these novel compounds truly come from.

Vascular plants rely on complex relationships with the microbial world. The microbes that form these relationships with host plants are known as endophytes. Endophytes often colonize the tissues of plants and form a symbiotic relationship with them. In many cases, both the plant and endophytes benefit from the relationship. This is known as mutualism.

Oftentimes, microbes produce compounds known as secondary metabolites that in turn benefit the host plant through these mutualistic relationships. Secondary metabolites are chemical compounds that are produced by an organism to provide some kind of benefit, but are not necessary for the survival of that organism. For example, these secondary metabolites might be antibiotic compounds that protect the plant against a bacterial infection. Though the compound helps the plant, the plant does not absolutely need it for survival.

These secondary metabolites are an important source of novel bioactive compounds that may potentially be developed into therapeutics. As the collective understanding of secondary metabolites grows, the more researchers can begin to target these compounds. Understanding the production of secondary metabolites may also help researcher identify whether or not the isolated novel compounds have come from the plants itself or its symbiotic microbes.

The more that we understand about the microbial world, the more researchers can explore new organisms for novel compound isolation. In order to understand the

extent at which researchers are currently exploring natural sources for new compounds, it is important to first categorize the sources from which previously isolated compounds have come from.

My research focuses on identifying and classifying the sources of all novel compound isolations published in the American Chemical Society's *Journal of Natural Products* from 2015 to 2017. First, I recorded the kingdom and phylum of each natural product source. Then I recorded the biome from which the natural product source was taken from. The different biomes were classified as terrestrial, marine, freshwater, or microbial. Currently, 404 articles have been analyzed. This includes articles from the entire 2015 year and half of the 2016 year.

The results were then graphed and analyzed in order to see if there were any trends within where these natural products are coming from. If the majority of the novel compounds are coming from organisms of the same kingdom or phylum, this may indicate that researchers are only targeting specific organism classifications for novel products. Researchers may be isolating the majority of the novel compounds from a certain phylum, which may indicate that they need to expand their scope to include other phyla.

I hope that this project can serve as a guide for researchers at other institutions that are attempting to discover novel compounds from natural products. This research can help act as a guide that reveals where current researchers have been searching for new bioactive compounds and where they have not yet isolated new bioactive compounds from. In order to develop a full understanding about the novelty of the

isolated compounds, I am working with Dr. John D'Angelo (Alfred University), Dr. Geoffrey Lippa (Alfred University), and Dr. Stephanie Rugg (Alfred State College). Through combining all of the data, we will have developed a comprehensive review of novel natural compound sources, bioactivity, and chemical structures.

Introduction

Microbial antibiotic resistance is a growing global public health issue. Antibiotic resistance occurs when bacteria develop the ability to resist antibiotic medications that would otherwise kill them or inhibit their growth. Developing genes that allow for resistance against antibiotics puts these bacteria at a natural advantage over non-resistant bacteria. Repeated exposure to antibiotics allows antibiotic resistant bacteria to survive and multiply, eventually helping the antibiotic resistant bacteria to outcompete non-resistant bacteria.

The repeated exposure to antibiotics can be linked to the over-prescription by outpatient clinics, the misuse of antibiotics by patients, and the overuse of antibiotics in industrial livestock farming (Alanis, 2005). These strongly fuel the development of microbial antibiotic resistance. Repeated dosage of antibiotics can not only cause resistance within the host organism, but microdoses of antibiotics can enter water ecosystems through host urine and feces. Once in a water environment, the environmental bacteria are also exposed to antibiotics; thus, bacteria in the environment can begin to develop antibiotic resistance before even infecting a host (Alanis, 2005). This ultimately amplifies the number of antibiotic resistant bacteria in the environment.

Infections by antibiotic resistant bacteria may be more severe, and are often more difficult to diagnose and treat overall. Treating infections caused by antibiotic resistant bacteria requires the use of new and stronger antibiotics. Bacterial antibiotic resistance is known to occur against several classes of antibiotics. Current antibiotics are split into two classifications by how they affect the target bacteria. (Kohanski, 2010) These classifications are bactericidal antibiotics and bacteriostatic antibiotics. Bactericidal antibiotics cause microbial death while bacteriostatic antibiotics hinder microbial growth. Microbes may acquire antibiotic resistance against more than one classification of antibiotics at once. This phenomenon is known as “multi-drug resistance” and makes treating these particular bacterial infections extremely difficult (Kohanski, 2010).

In order to offset the emergence of drug-resistant bacteria, the pharmaceutical industry and large academic institutions must devote significant research towards discovering new antibiotic compounds. Modification of the known chemical structures of antibiotics does not produce enough effective and safe novel antibiotics to keep up with the increasing levels of antibiotic resistance. Therefore, researchers must turn towards novel natural product sources in order to isolate new biologically active compounds. If there are more novel structures, there are more likely to be more novel mechanisms of actions. This increases the chances of generating new therapeutics.

Natural product sources such as plants and fungi have been employed as traditional herbal medicine far before modern drug discovery. Since the 19th century, these natural sources including plants, fungi, bacteria, and animals have been utilized in

the development of many important antibacterial, anti-inflammatory, and antitumor medicines (Zhang, 2006). Not only can therapeutic compounds be directly isolated from natural product sources, but natural product sources also provide template compounds for the laboratory synthesis of new drugs. To date, upwards of 70,000 vascular plant species have been evaluated for potential medical use. In 2000, the World Health Organization stated that 11% of the 252 ‘basic and necessary’ drugs were sourced from flowering vascular plants (Zhang, 2006). Though a great portion of the isolated therapeutic natural products have come from plants, new research reveals plant-associated and animal-associated microbes are important natural product sources as well.

Within the field of ecology, we understand that various organisms interact with the physical, chemical, and biological elements of their environment. These interactions collectively form what is known as an ecosystem. Though we can view large-scale ecosystems with the naked eye, there is a whole unseen world thriving around us. Thousands of species of bacteria, archaea, protists, and fungi interact with one another to form complex ecosystems known as microbiomes. These microbiomes exist on virtually every surface and even play important roles in the internal environments of other organisms (Jensen and Fenical, 1994). A well-known example is the human gut microbiome. In 2008, the Human Microbiome Project set out to collect data on the various complex microbiomes that exist within our own bodies. As the understanding of our own microcommunities grows, researchers are identifying and characterizing

microbiomes within other organisms including vascular plants, marine sponges, and coral (Jensen and Fenical, 1994).

The Plant Microbiome

Endophytic microbes are microorganisms that colonize the inside of plants. These microbes form symbiotic relationships with plants through living in close proximity to plant cells. In particular, the collective understanding of endophytic microbiomes has increased significantly over the past thirty years. Understanding the interactions between endophytic fungi and their vascular plant hosts can provide insight into the metabolic processes of both organisms.

Though some information is known about endophyte biology, not much is known about the evolutionary ecology of these microorganisms or how the relationships with their host plants first formed. It is hypothesized that the symbiotic relationships between endophytic fungi and higher plants have existed since the appearance of vascular plants (Rodriguez, 1997). It is believed that some endophytes first entered the plants through wounds in their dermal tissue, while some entered the xylem of the plant through the root tissue. The microbes then adapted to the tissue microenvironments of the host plant, forming relationships with their hosts.

Relationships between organisms can occur in a variety of different ways. Relationships between endophytic microbes and their plant hosts are often either mutualistic, commensal, or parasitic. Mutualistic relationships form when both organisms benefit from interacting with one another. Commensal relationships occur when one species benefits while the other in unaffected by the interaction. Finally,

parasitic relationships occur when one species benefits while the other is harmed by the interaction.

Though the vascular plant is often referred to as a ‘host’, this does not necessarily imply a parasitic relationship between the plant and the microbe. Endophytic microbes often produce chemical compounds known as secondary metabolites. Secondary metabolites are compounds that are not necessary for the survival of the organism but may still be beneficial to its overall success. For example, producing bioactive secondary metabolites may help the organism resist pathogens. Though these metabolites help the organism, the organism would still be able to exist without producing them.

The production of secondary metabolites by endophytic microbes plays a critical role in the formation of mutualistic relationships between microbes and their host. For example, some endophytes are known to incorporate host plant DNA into their own genome, allowing the endophyte to synthesize secondary metabolites that are beneficial to the success of the host plant. Supporting the host in this way would in turn be advantageous to the endophyte as the host provides protection. A strong, successful host plant insures that the endophytes have an environment to thrive in.

Though the general benefits of endophytic secondary metabolite production are known, the exact interactions between the endophyte and its host plant are not completely understood. Oftentimes, symbiotic interactions are extremely complex and can occur in a variety of different ways. On one hand, the host plant can also influence secondary metabolite production by the endophyte. On the other hand, the presence of

an endophyte may trigger a metabolic response in the host plant. This often occurs when the endophyte is pathogenic and its presence induces a host immune response. It is important to consider how the effects of different plant-endophyte relationships may influence endophyte secondary metabolite production as different plant-endophyte relationships result in the production of unique secondary metabolites.

Unique tissue microenvironments also allow for the colonization of different microbes and the production of different secondary metabolites. Oftentimes, the endophytes colonize specific tissue environments in the host plant rather than colonizing all tissue types. Microbes are known to colonize both the endosphere and the rhizosphere (Hamblin, 1986). The endosphere refers to the internal microenvironment of the plant while the rhizosphere refers to the microenvironment around the root system.

The physical structures of the host plant itself impacts the microbiomes within and around these plant structures. In order to demonstrate this, Saleem et al (2016) compared the abundance of different endophyte taxa across a root gradient from fine roots to primary roots within plants in the *Nicotiana* genus. It was determined that three species of Actinobacteria decreased in abundance from the outermost root hairs to the primary root structure. The increased surface area on the outermost fine root hairs allows for better nutrient availability and more colonization opportunities than the primary root structure. These data indicate that microbial ecosystems both within and around the plant can differ significantly despite existing in close proximity. This reveals the specificity of the various microenvironments and complexity of the microbiomes within them.

The different rhizosphere and endosphere microenvironments can be influenced by both physical and chemical factors. For example, soil compaction may impact the range of plant root growth (Alameda and Villar, 2009). Soil texture also influences root growth. A high number of transmission pores within soft soil allows for better water and nutrient movement. Loamy, nutrient-rich soil contains many transmission pores that not only move water towards the plant's root system, but these pores can also act as pre-existing channels for root hair expansion (Hamblin, 1986). Higher nutrient and water availability lead to increased root growth.

Chemical composition of the soil also plays an important role in root growth. For example, outer root structures are more sensitive to nitrogen levels than primary root structures (Tian et al, 2014). Soil conditions, like nitrogen levels, not only directly affect root growth, but they also indirectly affect the microbiomes in and around these root structures. Thus, endophyte colonization and microbiome makeup are directly related to the physical structures of the host plant.

In many cases, each host microenvironment is so specific that it can be considered a different ecosystem. Unique conditions of the distinct microenvironments within different areas of the plant can influence secondary metabolite production and overall success of the endophyte species. This specificity is a result of endophyte and host plant adaptations. Not only does the host plant environment benefit non-pathogenic endophytes, but the presence of the endophytes may benefit the host as well. These endophytes may be directly involved in synthesizing entire or partial chemical compounds that are utilized by the host plant (Ludwig-Muller, 2011). These complex

interactions help fuel the symbiotic relationships between the endophytes and their host plant, aiding in the ultimate success of both species.

In order to exemplify these symbiotic chemical interactions, Hallman and Sikoura (1996) isolated strains of the non-pathogenic endophytic fungi *Fusarium oxysporum* from tomato root cortical tissue. Culturing this fungi in nutrient-rich broth led to the production of secondary metabolites that are toxic to the nematode *Moloidogyne incognita*. This parasitic nematode attacks the host plant through the root system and degrades the plant for nutrients. Hallman and Sikoura (1996) exposed juvenile and adult nematodes to the cultured endophytic fungi. The metabolites produced by the cultured endophytic fungi resulted in 100% juvenile nematode mortality after 24 hour exposure.

Hallman and Sikoura (1996) then conducted a bioassay using lettuce seedlings and determined than the *F. oxysporum* secondary metabolites were only effective against parasitic nematodes but were not effective against non-parasitic nematodes. It was also shown that the secondary metabolites significantly reduce the growth of several other plant pathogens including *Phytophthora cactorum*, *Pythium ultimum*, and *Rhizoctonia solani*. This demonstrates that the secondary metabolites produced by the endophytic fungi are beneficial to the host plant by protecting it from parasitic damage.

The Sponge Microbiome

Another important source of natural products is the marine sponge. The sponge microbiome is comprised of an intricate and diverse mix of bacterial, archaeal, and eukaryotic symbionts that can contribute to up to 35% of the sponge's overall biomass. Specifically, sponge-associated bacteria from 28 different phyla have been identified

through 16 S rRNA sequencing- a phylogenetic analysis method that compares unknown bacterial gene sequences to an established library of bacterial gene sequences (Thomas, 2016). The most abundant bacterial phyla include Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria, Nitrospirae, and Poribacteria. Interestingly, the Poribacteria phylum contains bacteria that are only found within marine sponge microbiomes. Other low abundance bacterial phyla have been discovered through advanced sequencing techniques as well (Thomas, 2016). The sponge bacterial microbiome is considerably diverse when compared to other animal microbiomes. To put this into perspective, the human gut microbiome is comprised of two dominant bacterial phyla, Firmicutes and Bacteriodes, which is considerably less than the number of dominant bacterial phyla in the marine sponge microbiome (Thomas, 2016).

Recent data suggest that sponge-associated bacteria have a high degree of host specificity, meaning that most of these bacterial species are only associated with a few host sponge species. This implies that the host sponge species are able to select certain symbionts to remain in their microbiome, rather than relying only on natural selection pressures to determine their microbiome makeup. When considering sponge physiology, this selective ability makes sense. As filter feeders, sponges draw in water, bringing a variety of microorganisms into their inner connective, or mesohyl, tissue. As these microorganisms interact with the mesohyl tissue, it is important for the sponge to recognize which microbes are food and which microbes are symbionts. Sponges also produce bioactive antimicrobial compounds as part of their immune system and must be

able to target harmful microbes rather than their own symbionts. This recognition ability allows the sponge to maintain a symbiotic microbial community within itself.

The sponge microbiome is comprised of a mixture of heterotrophic and autotrophic microorganisms. Heterotrophic microbes utilize organic matter as their main source of carbon, while autotrophic microbes utilize carbon dioxide for their main source of carbon. Sponges also provide the symbionts with necessary nitrogen through ammonia and nitrite waste products. Low levels of urea from the ambient water drawn into the sponge when filter-feeding may also act as another source of nitrogen for the symbionts. Not only does the efficient recycling of nitrogenous waste products benefit the host sponge, but recent genomic data indicate that the sponge-associated microbes may synthesize B vitamins (B_1, B_2, B_6, B_7, and B_{12}) as well. The vitamins produced through these metabolic processes may be taken up and utilized by the sponge in order to supplement its vitamin B nutrient requirement (Proksch, 1994). Ultimately, the metabolic processes of the microbes support and drive the symbiotic relationship with the sponge host.

Sponges establish their complex microbiome through two main methods: through vertical transmission from parent to offspring or through horizontal transmission by recruiting microbial symbionts from the ambient water (Proksch, 1994). Data indicating that microbiome composition is similar within different stages of sponge maturity reveals that a large portion of the sponge microbiome is inherited through vertical transmission. Vertical transmission results in the maturity of the sponge and its microbiome together

with no aposymbiotic phase in development. An aposymbiotic phase is a stage in organismal development in which the organism contains no symbiotic microbiome.

This mode of symbiont transmission allows the sponge to coevolve with its microbiome. The coevolution of the sponge and its symbionts can be observed through the loss of sections of the symbiont genome. As the host coevolves with the microbes, the microbes will lose genes that would allow them to exist outside of the sponge host (Moitinho-Silva, 2017). This shows that the symbiotic microbes truly become a part of the host organism.

Though there is a collective understanding of how the sponge coevolves with its microbiome, the question of why is still being explored. Living within the sponge not only allows the microbes to be protected from various harmful environmental factors, but the microbes are constantly supplied with nutrients as the sponge filter-feeds. In order for environmental pressures to push microbes towards coevolving with the sponge, living within the sponge must be more beneficial than living outside the sponge.

A crucial link between microbes and their host sponge is the production of eukaryotic-like proteins. When produced by the bacteria, these proteins help mediate host metabolic processes and support the coevolution of the sponge and its symbionts. Genes associated with the production of these eukaryotic-like proteins have been identified in two different sponge-associated bacterial genomes (Moitinho-Silva, 2017). This may be due to the incorporation of host genetic material into the genome of the symbiont. The eukaryotic-like genetic motifs may help the symbiont remain protected against the sponge's immune system. The protein structural motifs may make detection

of the symbiont difficult. Specifically, genes identified from Poribacteria may be linked to the production and modification of sponge extracellular matrix proteins including proteoglycans, glycoproteins, collagen, and spongin (Moitinho-Silva, 2017). These proteins influence the structure of the sponge as well as the communication between cells. A form of communication between bacterial cells known as quorum sensing is also utilized by several sponge-associated symbionts. This method of communication between symbiont cells may also be used to relay information between symbionts and host cells. The production of these eukaryotic-like proteins allows for close interaction between the host sponge and its symbionts.

The ability to fully study the exact functions of sponge-associated microbes is hindered by the difficulty of culturing symbionts outside of the sponge host. The complex series of symbiotic interactions between the microbes and their sponge ecosystem allows them to develop and reproduce successfully. Since a successful experimental model system that allows for similar ecosystem interactions does not yet exist, it is difficult for these microbes to grow and perform the same way that they would inside the host. However, the roles of these microbes can be better understood using genomic and proteomic analysis to identify their metabolic processes. Identifying the symbionts' contributions to the sponge microbiome allows for greater knowledge of microbe-microbe and microbe-sponge interactions, as well as an increased understanding of the sponge-microbiome symbiotic relationship.

Besides producing eukaryotic-like proteins that interact with the sponge's metabolic pathways, sponge-associated symbionts also produce secondary metabolites

that are utilized by the sponge. These secondary metabolites may be beneficial to the success of the sponge as chemical defense mechanisms against pathogens. Originally, it was thought that these compounds are only produced by the sponge; however, it is now understood that these secondary metabolites compounds are produced by both the sponge and its symbionts. Understanding that secondary metabolites within the sponge ecosystem come from different sources broadens the range of bioactive natural compound sources. Rather than looking at the sponge as one whole organism producing natural compounds, it is now important to look at the many organisms within the sponge microbiome as well. Ultimately, this knowledge increases the chances of isolating and identifying novel bioactive compounds from marine organisms.

In order to truly understand the trends in novel natural product isolation, reviewing and categorizing the sources of novel natural products would be extremely beneficial. This study aims to categorize the sources of novel natural products published in the American Chemical Society's *Journal of Natural Products* in 2015. Ultimately, we hypothesize that scientists are attempting to isolate novel natural products with too narrow of a focus. This research quantifies and categorizes the sources of novel natural products in order to reveal if scientists are identifying novel compounds from only specific taxonomic groups or habitats. We hypothesize that the majority of novel natural products have been isolated from the Plantae kingdom, specifically from the Tracheophyta (vascular plant) phylum. We also hypothesize that the majority of novel natural products have been isolated from the tropical rainforest biomes rather than other terrestrial or marine biomes.

Methods

In this study, 404 articles from the *Journal of Natural Products* 2015 year were reviewed and classified into various categories including: novel compound isolation with structure identification, novel compound isolation without structure identification, structure identification of a known compound, structural revision of a known compound, new biological activity of known compound, methodology, synthesis, derivative synthesis, or other. Both types of isolation papers were then studied further in order to identify the natural source of the newly isolated compound.

Both the kingdom and phylum of each isolated compound were recorded. Habitat (biome) was also recorded for each non-microbial natural source species, while microbiome was recorded for each microbial natural species. The different biome classifications included 11 terrestrial biomes, 10 marine biomes, 3 freshwater biomes, and 14 microbiomes (Table 1).

Table 1. Terrestrial biome, marine biome, freshwater biome, and microbiome categories

Terrestrial	Marine	Freshwater	Microbiome
Boreal Forest/Taiga	Abyssal Plain	Lakes	Algae
Desert	Coral Reef	Ponds	Animal Dung
Grassland	Deep Sea	Rivers	Arthropod
Savanna	Kelp Forest		Cnidarian
Scrub/Woodland	Mangrove Forest		Freshwater Sediment
Seaside/Beach	Open Ocean		Fungi
Temperate Forest	Polar Ocean		Marine Sediment
Temperate Rainforest	Rocky Shore		Marine Sponge
Tropical Rainforest	Saltmarsh/ Mudflat		Nematode
Tropical Seasonal Forest	Sandy Shores		Saltern/Saltmarsh
Tundra			Terrestrial Cave
			Terrestrial Plant
			Terrestrial Rock Surface
			Terrestrial Soil

The percentages of natural sources from each kingdom were plotted in order to analyze whether novel compounds have been isolated from each kingdom equally. This then was repeated with phyla in order to analyze whether novel compounds have been isolated from each phylum equally. The counts from each biome and microbiome were then plotted to analyze which biome/microbiome had the highest recorded number of natural sources.

Results

An analysis of the percentage of novel compounds isolated from each taxonomic kingdom (Animalia, Plantae, Fungi, Protista, Eubacteria, and Archaebacteria) revealed that 66.5% of novel compounds had been isolated from species in the Plantae kingdom. Less novel compounds had been isolated from Fungi (19.7%), Animalia (7.1%), and Eubacteria (6.7%), while no novel compounds had been isolated from Protista or Archaebacteria (Figure 1). These data indicate that researchers may be relying too heavily on plant species to provide novel natural products; and it may be beneficial to shift the focus from Plantae to the other taxonomic kingdoms.

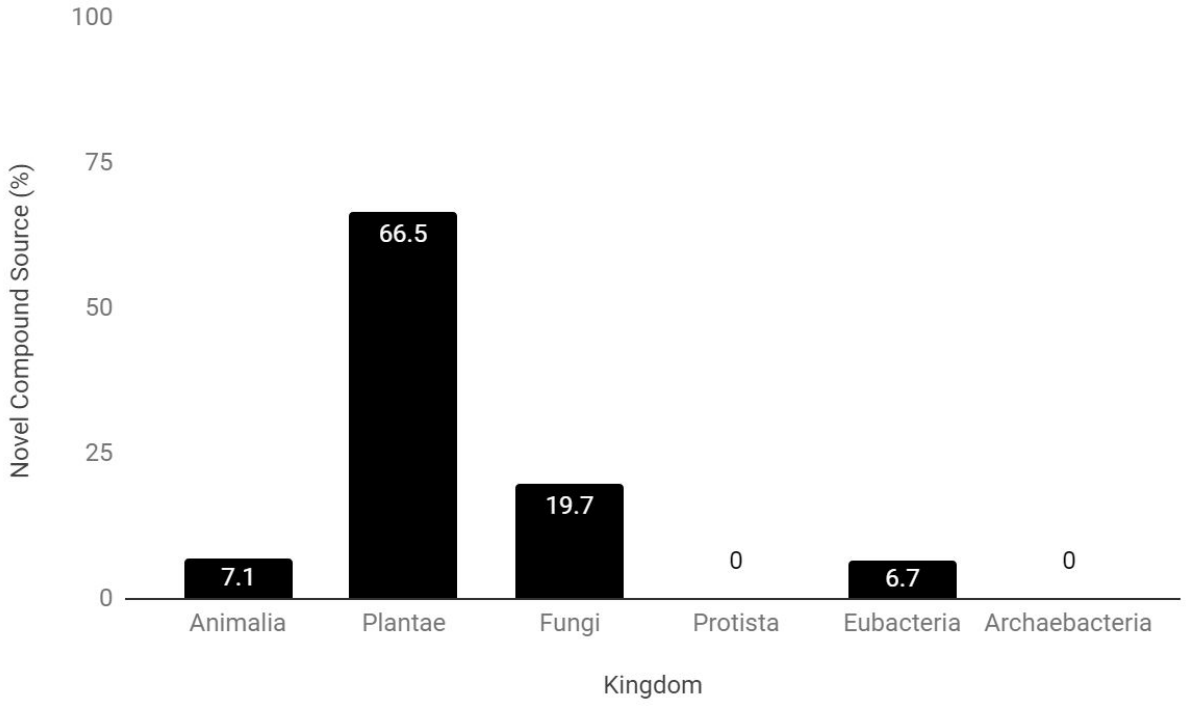

Figure 1. Percent of novel compound sources from each taxonomic kingdom (Animalia, Plantae, Fungi, Protista, Eubacteria, and Archaebacteria).

When analyzing these data by phyla, it was determined that 53.7% of all novel compounds isolated in 2015 came from species in the plant phylum Tracheophyta. The fungi phylum Ascomycota followed with 18.4%, and the eubacteria phylum Actinobacteria then followed with 7.4% (Figure 2). This indicates that the majority of newly isolated compounds in 2015 have originated from vascular plants (Tracheophyta).

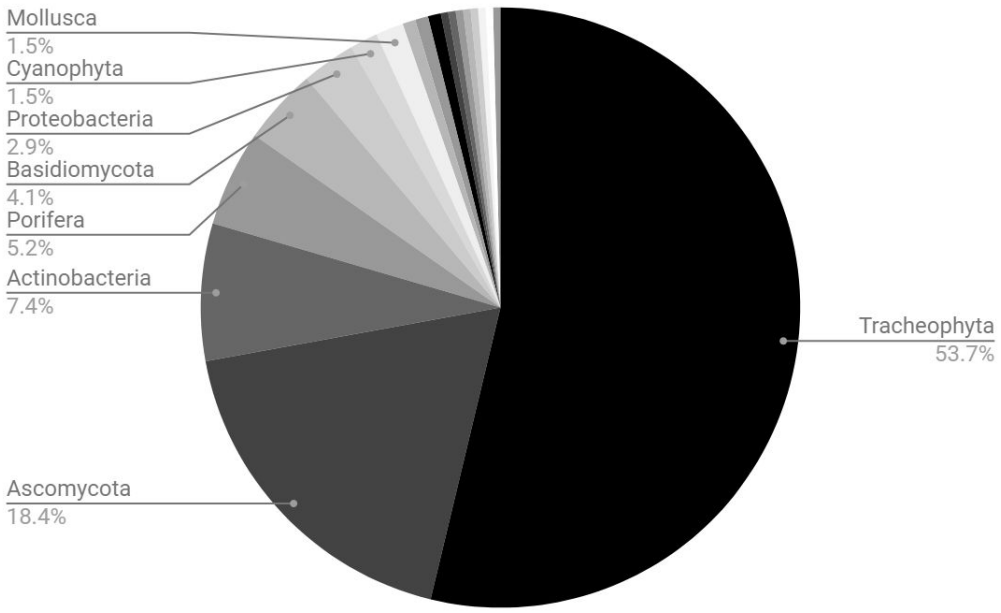

Figure 2. Percentage of novel compound sources from each recorded taxonomic phylum.

An analysis of the total novel compound sources from terrestrial, marine, freshwater, and microbial habitats reveals that the majority of novel compounds had been isolated from species residing in terrestrial biomes (161 total species), particularly in the tropical rainforest and temperate forest (79 and 54 total species, respectively) (Figure 3). These data also revealed that far less compounds had been isolated from species residing in marine biomes (42 total species) with 25 of those species originating

from coral reefs. Only 2 total species from freshwater biomes yielded novel compounds, while the various microbiomes were the sources of only 64 total species (Figure 3). The one-way ANOVA indicated that there was a significant difference between the terrestrial, marine, freshwater, and microbiome habitats (p=0.028).

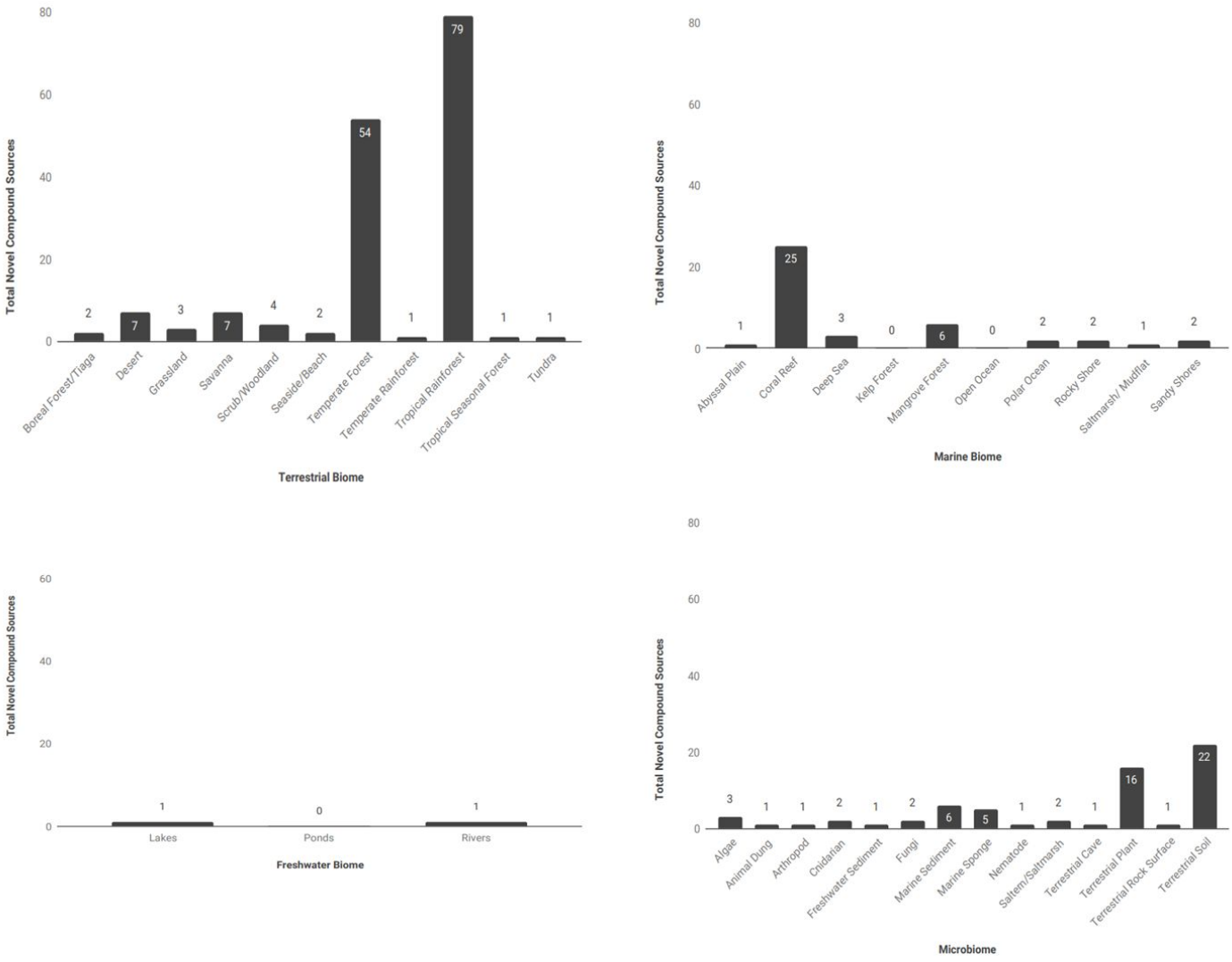

Figure 3. Total number of novel natural product sources in each biome (terrestrial, marine, freshwater, and microbial).

Discussion

The data indicate that the majority of the isolated compounds have come from the kingdom Plantae (66.5%) which suggests that there may be too narrow of a focus on isolating novel compounds from plants, specifically from the Tracheophyta phylum. Some kingdoms remain rather unexplored including Protista and Archaebacteria. This may be attributed to simply not exploring these kingdoms, or difficulty extracting novel compounds from these sources.

In terms of biome characterization, 79 novel compounds were isolated from the tropical rainforest and 54 were isolated from the temperate forest. The majority of compounds were isolated from terrestrial biomes while only 2 compounds were isolated from freshwater biomes.

Ultimately, these data indicate that natural product researchers may need to expand their focal point when searching for new natural product sources. In order to continue developing therapeutics that combat global public health issues like antibiotic resistance, researchers may need to shift towards unexplored taxonomic groups and habitats, particularly microbial species, for novel bioactive compounds.

In the future we plan to complete a full analysis of all *Journal of Natural Products* articles from 2015 through 2017. Not only will we analyze these articles for novel compound sources, but we will also be analyzing the compounds themselves in order to determine if there are frequently recurring chemical motifs among these novel compounds. We will also be recording bioactivity of these novel compounds in order to determine if there are more novel compounds isolated with similar bioactivities. Overall,

we seek to quantify all possible natural product sources and possibly call attention to untapped novel natural product sources. This work may act as a guide for natural product researchers and may help pave the way for novel natural product isolation and the development of new therapeutics.

References

Alanis AJ. Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res. 2005; 36: 697-705.

Alameda D and Villar R. Moderate soil compaction: implications on growth and architecture in seedlings of 17 woody plant species. Soil Till Res. 2009; 103: 325-331.

Alvin A, Miller KI, and Neilan BA. Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. Microbiol Rev. 2014; 169: 483-95.

Baquero F, Martinez J, Canton R. Antibiotics and antibiotic resistance in water environments. Curr Opin Biotechnol. 2008; 19(3): 260-65.

Hamblin AP. The influence of soil structure on water movement, crop root growth, and water uptake. Adv Agron. 1986; 38: 95-158.

Hentschel U, Piel J, Degnan SM, and Taylor MW. Genomic insights into the marine sponge microbiome. Nat Rev Microbiol. 2012; 1: 1-4.

Jensen PR and Fenical W. Strategies for the discovery of secondary metabolites from marine bacteria: ecological perspectives. Annu Rev Microbiol. 1994; 48: 559-84.

Journal of Natural Products. 2015-2017. All articles.

Kohanski MA, Dwyer DJ, and Collins JJ. How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol. 2010; 8(6): 423-35.

Ludwig-Muller J. Auxin conjugates: their role for plant development and in the evolution of land plants. J Exp Botany. 2011; 62(6): 1757-1773.

Moitinho-Silva et al. The sponge microbiome project. Gigascience. 2017; 6: 1-7.

Proksch P. Defensive roles for secondary metabolites from marine sponges and sponge-feeding nudibranchs. Toxicon. 1994; 32(6): 639-55.

Saleem M et al. Impact of root system architecture on rhizosphere and root microbiome. Rhizosphere. 2018; 6: 47-51.

Tian H, Smet ID, and Ding Z. Shaping a root system: regulating lateral versus primary root growth. Trend Plant Sci. 2014; 19: 426-431.

Thomas T et al. Diversity, structure and convergent evolution of the global sponge microbiome. Nat Commun. 2016; 1: 1-10.

Webster NS et al. Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. Environ Microbiol. 2010; 12(8): 2070-82.

Zhang HW, Song YC, and Tan RX. Biology and chemistry of endophytes. Nat Prod Rep.
2006; 23: 753-71.