Bacterial Species Biodiversity in the Concha and Belly Button of Humans

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Introduction

Biodiversity can be defined as the variability of living organisms from all sources and the ecological complexes they are a part of, such as diversity between species and ecosystems, and within species (Titley et al., 2017). Most diversity is discussed at the species level, as that is the complex that correlates best with species richness. Species richness on the other hand is the number of different species that are present in a specific area. Species richness is a good measure of species biodiversity because it provides researchers or scientists with a measure of how assorted a population is. Species richness across regions can only be changed and explained based on speciation, extinction, and dispersal (Pyron and Wiens, 2013). Once there is a general idea of how many different species there are present in the region of study, species' abundance can be observed. Species' abundance is a measure of how many individuals there are from one species relative to the entire population. These concepts can be used to understand complex habitats and provide reasons for varied biodiversity across numerous regions such as the tropics or temperate zones.

Tropical regions tend to be humid, wet, and consist of savannahs and rainforests (Romer, 2013). They tend be located at lower latitudes near the equator where there are large amounts of rainfall, high temperatures, an abundance of water, and products of biological decay (Romer, 2013). On the other hand, temperate regions are defined as having moderate annual temperatures and tend to have four distinct seasons throughout the year (Pratolongo et al., 2019). Tropical environments are said to be more stable and are associated with stable climatic factors, while temperate environments consist of a wider range of climates that can vary based on the time of the year and are found in more locations (Romer, 2013; Pratolongo et al., 2019). Due to the varying levels of stability that the two environments can provide, it creates questions as to which regions are prone to more speciation events and which regions can support a larger biodiversity.

Biodiversity varies from region to region and a major theme within is the debate of why species diversity is higher in tropical regions compared to temperate regions. There are over 100 tested hypotheses that take this question into account and try to explain this phenomenon (Wiens et al.,, 2006). A major idea that involves numerous hypotheses is that tropical regions allow for ecological processes that provide coexistence of large numbers of species (Wiens et al., 2006). With this, is the idea of understanding environmental variables that coincide with patterns of increased speciation rates which then leads to higher biodiversity (Wiens et al., 2006). Two major hypotheses, the energy and productivity hypothesis, support this idea for tropical regions in which the climate reinforces high primary productivity which supports more individuals which can lead to higher species diversity (Lawrence and Fraser, 2020). The tropics are located at lower latitudes compared to temperate regions and with that comes a more stable environment in the tropics with less climatic variability. Since the climate is less variable, species are adapted to a small niche range which makes dispersal difficult (Lawrence and Fraser, 2020). With low dispersal rates, species tend to remain in the region they are adapted to, which keeps species within the tropics, while species in temperate regions are more likely to dispersal outward. When immigration rates outweigh migration rates, which occurs in the tropics, species accumulate in that region which increases biodiversity (Lawrence and Fraser, 2020). Also, biotic interactions are more prominent in the lower latitude tropic regions and create speciation while abiotic interactions are more prominent in the higher latitude temperate regions (Lawrence and Fraser, 2020). The more speciation that takes place in the tropic regions is yet another explanation for the higher biodiversity. In this paper we are using the human body as a model in trying to test the hypothesis that tropic regions yield more species diversity compared to temperate region. We are identifying the belly button as a tropic region and the concha of the ear as a temperate region and determining which site contains a higher bacterial species diversity.

The human body is a petri dish for many different types of bacteria. Depending on the area of the body, the amount of bacteria present can vary drastically (Evans et al., 1950). One area of continued interest is the belly button as it is an ideal environment for bacteria accumulation yet has not been thoroughly explored (Hulcr et al., 2012). Much like tropical regions, the belly button can be classified as a 'wet' environment which can be home to a wide diversity of bacteria. In a study that tested bacterial species of human belly buttons, it was found that frequent species tend to be common when present and more clustered, compared to rare species (Hulcr et al., 2012). Also, it was found that the belly button was dominated by six main bacterial species although those species only represented a small fraction of the total human bacteria found (Hulcr et al., 2012). One reason that might explain the idea of the common species could be that over time they have evolved and are now predisposed to be able to survive in the environments they reside (Hulcr et al., 2012). This can add to the fact that species present in tropical regions don't tend to disperse which can be predicted of bacterial species in the belly button. We can think of the human body as a globe with varying latitudes and the waistline being the equator. This would mean that the belly button is at a low latitude, which is consist with the tropics, and could support the idea of the belly button having a high level of bacterial species diversity. The more thought that goes into thinking of the belly button as a tropical region, the more the belly button resembles a region in which bacterial species can diversify.

Using the human body as a means in comparing natural environments, we believe the concha of the ear can easily resemble a temperate environment. The concha of the ear is the region that opens the external auditory canal and is not covered by the pinna, or the flap of the ear. Although the ear might be a region of humidity and moisture, the concha is not, as it is opened to the external environment with no protection. The concha can be classified as being a 'dry' environment which is synonymous to a temperate region with varying bacterial species presence (Hulcr et al., 2012). There is no previous research that analyzes the bacterial species present in the concha of the ear, so looking at human skin bacteria, evidence shows that areas that are more exposed, have more varying bacterial species (Evans et al., 1950). Also, the more exposed an area is, such as the concha, the more dirt and external factors affect the present bacteria (Evans et al., 1950). Since no research has been done, we do not know if the increased exposure increases species diversity, or just allows for continual movement of bacteria in and out of the area. This idea could help us predict that species present in the concha could be more willing to disperse outward. Thinking back to the human body as a globe with varying latitudes, the concha resides at a high latitude which is more consistent with temperate regions and with varying levels of species diversity.

We hypothesize that a tropical environment such as the belly button will yield higher bacterial species diversity compared to that of a temperate environment such as the concha of the ear. To test this, we are sampling the concha and belly button of 20 individuals and comparing the varying levels of bacterial species biodiversity. Analysis will be achieved at multiple levels to determine which sample site has more species present, more abundance of species, and more species diversity.

Methods

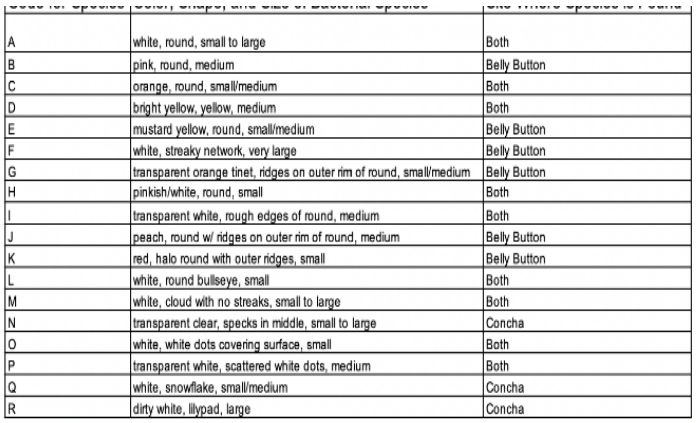
On April 2, 2022, from 3:55pm to 4:06pm, bacteria sampling was taken from both the belly button and the concha of the ear of 20 male hockey players from Lake Forest College. We performed all the sampling, and we plated all the bacteria.

A dry sterile Q-tip was turned around the belly button of an individual three times with constant pressure. The Q-tip was then stirred in a 0.5 mL tube of PBS for 5 seconds to ensure thorough mixing and then the Q-tip was discarded. A new dry sterile Q-tip was used and was turned around the concha (of the ear) three times with constant pressure. The Q-tip was then stirred in a new 0.5 mL tube of PBS for 5 seconds to ensure thorough mixing and then the Q-tip was discarded. Once an individual had their belly button swabbed, their concha was swabbed directly after before moving onto the next individual and the procedure was repeated for the remaining 19 individuals. The tubes were refrigerated after all sampling was completed for 44 hours and 30 minutes.

On April 4, 2022, at 12:30pm, we plated all the bacteria. The 0.5 mL tube containing the PBS in which the Q-tip was stirred, was shaken thoroughly and then 50 uL of the suspension was pipetted out and added to another tube containing 950 uL, to create a 1:20 dilution. The tube containing the

1:20 dilution was shaken thoroughly and then 50 uL was pipetted out and onto the surface of a pre-labeled TSA plate. 5-7 sterile glass beads were added to the TSA plate and the plate was rotated to spread the suspension over the entire surface of the agar. The beads were then dumped out of the plate and into a waste jar. This procedure was repeated for each sample. All the tubes were saved and refrigerated in case of plate errors. Once all the plates were completed, parafilm was wrapped around the edges to seal each one and then they were inverted and placed in a 360C incubator, which occurred at 2:00pm. The plates were first checked after 48 hours and every subsequent 24 hours until enough bacteria grew for analysis. After 7 days in the incubator, enough bacteria grew, pictures were taken of each plate, and plates were placed in a refrigerator to prevent further bacteria growth and to have as a resource. Each plate was analyzed for bacterial growth and all the different bacterial species were differentiated by color, shape, and size, and then given a letter code for easy referral.

A two-sample paired t-test for means was performed for the mean bacterial species richness present in the concha and belly button. The Shannon-Wiener diversity index was calculated for each sample and then the mean index value was calculated for the concha and belly button. A two-sample paired t-test for means was performed for the mean Shannon-Wiener diversity index value for the concha and belly button.



A	white, round, small to large	Both
B	pink, round, medium	Belly Button
C	orange, round, small/medium	Both
D	bright yellow, yellow, medium	Both
E	mustard yellow, round, small/medium	Belly Button
F	white, streaky network, very large	Belly Button
G	transparent orange tinet, ridges on outer rim of round, small/medium	Belly Button
H	pinkish/white, round, small	Both
I	transparent white, rough edges of round, medium	Both
J	peach, round w/ ridges on outer rim of round, medium	Belly Button
K	red, halo round with outer ridges, small	Belly Button
L	white, round bullseye, small	Both
M	white, cloud with no streaks, small to large	Both
N	transparent clear, specks in middle, small to large	Concha
O	white, white dots covering surface, small	Both
P	transparent white, scattered white dots, medium	Both
Q	white, snowflake, small/medium	Concha
R	dirty white, lilypad, large	Concha

Table 1: Table showing the 18 different bacterial species found in the concha of the ear and belly button of 20 sampled individuals. The species are identified by their color, shape, and size, and then the bacterial species are given a letter code for easy referral. The site in which the bacterial species was found is also recorded.

Results

18 different bacterial species were found between the belly button and the concha of the ear for 20 sampled individuals. We organized the bacterial species by color, shape, and size (Table 1). Of those 18 species, 6 were unique to just the belly button and 3 were unique to just the concha (Table 1). There was a lot of variability for the number of species found on each plate but the most species present on a single concha sample plate was 3, while for a single belly button sample plate it was 6 species. The average number of bacterial species present on the concha plates was 1.6 species while the average was 2.4 species for the belly button plates (Fig. 1). A paired two-sample t-test was performed for the average number of bacterial species present on concha versus belly button plates but it was not significant (t19 = -1.6895, p = 0.1075).

There was a total of 15 different bacterial species found in the belly button and 12 different bacterial species found in the concha (Fig. 2). Of the 18 total species, 12 species were found more in the belly button compared to the concha, 4 were found more in the concha compared to the belly button, and 2 were found the same in the concha and the belly button (Fig. 2). For both sample sites, the three species that were found on the most plates were species A, I, and D, and all three species appeared on more belly button plates compared to concha plates (Fig. 2). Of 20 belly button sample plates, there was only 2 plates that yielded no bacterial species and 5 plates that only had 1 bacterial species present (Fig. 3). While of the 20 concha sample plates 4 samples had no bacterial species present and 6 plates had only 1 species present (Fig. 3). This can show that 10 of the concha samples had no bacterial species diversity, while only 7 of the belly button samples had no species diversity.

There was a total of 467 bacterial species colonies present for the concha samples while there were 958 total bacterial species colonies found in the belly button samples. Of the 18 bacterial species, 13 were found to be more abundant in the belly button compared to the concha and 5 were found to be more abundant in the concha over the belly button (Fig. 4). We found that species D was one of the species that appeared on the most plates for both sites, but its abundance was quite small, being only the 9th most abundant species, having 17 total bacterial colonies present (Fig. 4). Species A was found on more belly button and concha plates compared to species I (Fig. 2), however, species I was more abundant than species A, 446 and 373 bacterial colonies, respectively (Fig. 4). Species J was found on 4 belly button plates (Fig. 2) but was not very abundant, only having 10 total bacterial colonies (Fig. 4) while species G was only found on 1 belly button plate (Fig. 2) but was more abundant, having a total of 73 bacterial colonies present (Fig. 4). Also, even though species N was only found on 1 concha plate (Fig. 2), it was abundant as it had a total of 47 bacterial colonies present (Fig. 4). Lastly, species O was found on 2 concha plates and 1 belly button plate (Fig. 2) buts its abundance was more drastic, having 109 bacterial colonies on concha plates and only 2 bacterial colonies on the belly button plate (Fig. 4).

Of the 20 sampled individuals, 9 had more bacterial species present in their belly button, 6 had more bacterial species present in their concha, and 5 had the same number of bacterial species present in their belly button and concha, although the diversity of those present species varied per individual. We calculated the average Shannon-Wiener diversity index for the diversity of bacterial species found in the concha and in the belly button of the 20 sampled individuals, 0.163 and 0.219, respectively (Fig. 5). No species diversity was found in the concha of individuals 2, 3, 4, 6, 10, 12, 14, 15, 16, 19 and the belly button of individuals 1, 5, 10, 15, 16, 17, 18, so those values were all zero. A paired two-sample t-test was performed for the average Shannon-Wiener diversity index value for the concha and belly button samples but it was also not significant (t19 = -1.563, p = 0.1346).

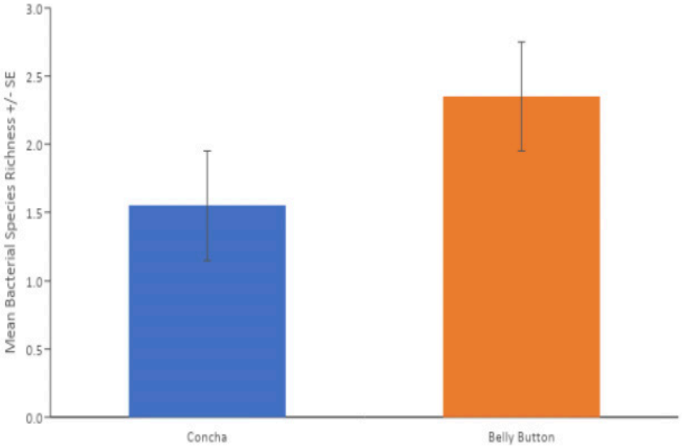


Figure 1: Bar graph comparing the average number of bacterial species found on all concha sampled plates compared to all belly button sampled plates of 20 individuals. The blue bar represents the concha while the orange bar represents the belly button. Black lines represent standard error.

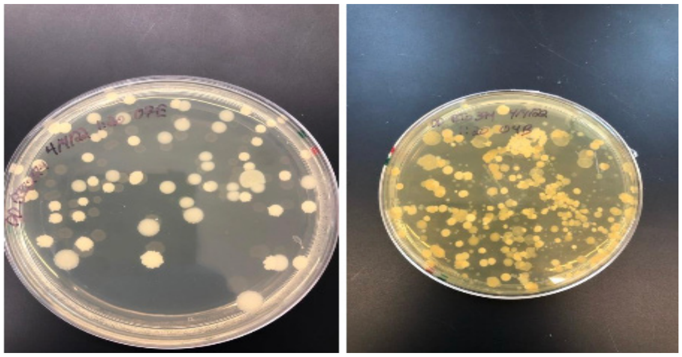


Image 1: These images show two examples of the petri dishes in which the bacterial species grew. The left image is sample 7 of the conchae while the right image is sample 4 of the belly button. All the present dots are bacterial colonies.

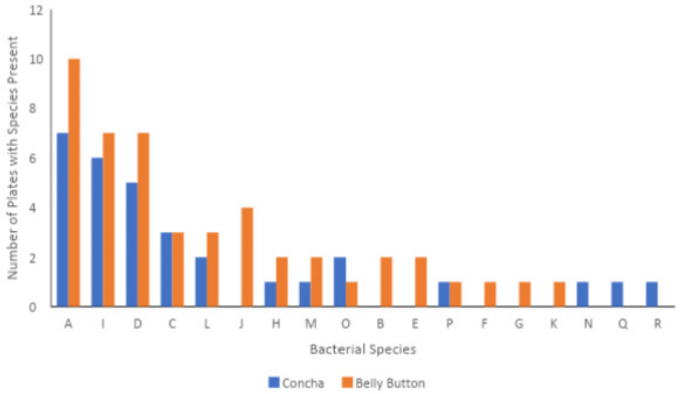


Figure 2: Bar graph showing the number of plates in which bacterial species were found in the concha of the ear and the belly button of 20 sampled individuals. The blue bars represent the concha while the orange bars represent the belly button.

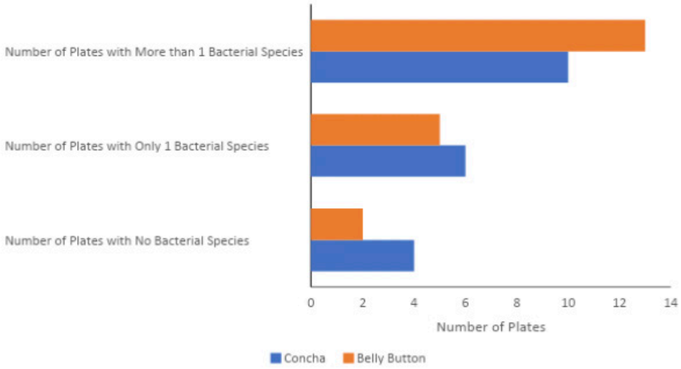


Figure 3: Bar graph comparing the number of belly button and concha plates of 20 individuals that had one or less bacterial species present and more than one bacterial species present. Blue bars represent the concha while orange bars represent the belly button.

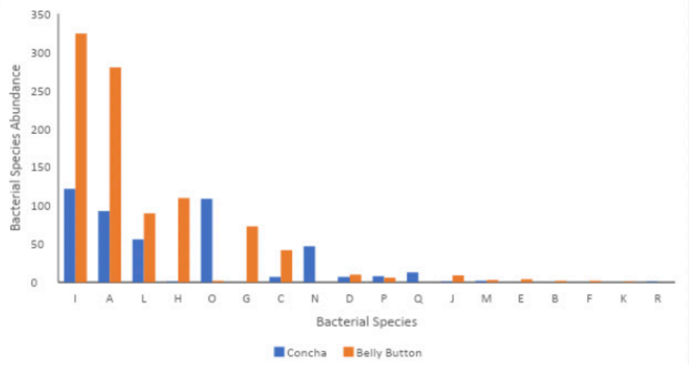


Figure 4: Bar graph showing the abundance of bacterial species found in the concha and belly button of 20 sampled individuals. Blue bars represent the concha while orange bars represent the belly button.

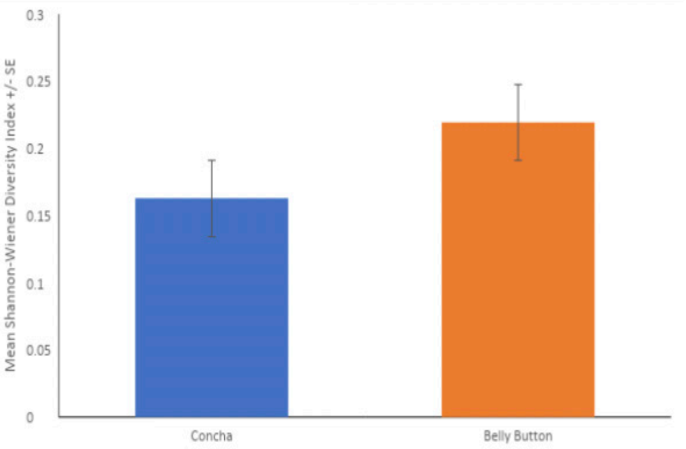


Figure 5: Bar graph displaying the average Shannon-Wiener diversity index value for the diversity of bacterial species present in 20 individuals' concha and belly button. The blue bar represents the concha while the orange bar represents the belly button. Black lines represent standard error.

Discussion

Based on our results, belly buttons have more bacteria and more species compared to the concha of the ear. This is consistent with our hypothesis in which we predicted the belly button, analogous to a tropical region, would have a larger bacterial species biodiversity compared to the concha, analogous to a temperate region. Belly buttons not only had more species present, but they also had a larger abundance of species and a higher species diversity (Fig. 4 and 5). Much like a tropical region, the belly button can be seen at a lower latitude, as a 'wet' environment, and has a more stable climate, so as a result, it had a higher bacterial species diversity compared to the concha. Much like a temperate region, the concha is at a higher latitude, is a 'dry' environment, and has a changing climate as it is more exposed to external factors compared to the belly button, so as a result, it was found it had less bacteria present and at a lower diversity. There was more bacterial species found to be unique to the belly button, so that can show the bacteria could be adapted to the belly button environment, whereas almost all the species found in the concha were also found in the belly button, which can show concha bacteria are more willing to disperse outward. Although we found our data to show the belly button has more bacterial species and more diversity of the species, the t-tests performed for the average number of species per plate and the average Shannon-Wiener diversity index, were not significant. This does not invalidate our findings, it just shows that the concha also has a significant number of bacterial species and diversity of those species relative to the belly button, just at a lower level.

Previous studies have shown that tropical environments have more species diversity compared to temperate environments, so we can use former hypotheses and former findings to explain what we found. Species found in the topics have narrow thermal breadths, decreased dispersal and higher population structure, and higher diversity and speciation rates (Polato et al., 2018). This idea can help explain why the bacteria we found in the belly button tended to stay in the belly button and became more abundant. Another idea from this is that more species could have been found in the belly button because overtime speciation could have occurred which created different looking bacterial species. Also, if bacteria that originated in the belly button dispersed all the way to the concha, they could have not been able to survive the different environment, which can explain the number of species found in the belly button and not in the concha. Other hypotheses that explain that the tropics are more stable, and the species found in the tropics have a small niche range, have more biotic interactions, and more diversification events (Lawrence and Fraser, 2020), help support our findings as the belly button represents a tropical region. Another thing we found is that even though there were more bacterial species in the belly button, the abundance was not always large, so species populations were somewhat small in the belly button. This idea is supported by the genetic drift hypothesis in which tropical species tend to have small population sizes which leads to genetic drift which creates speciation and hence, more species (Lawrence and Fraser, 2020). On the other hand, in a previous study that focused on plant genetic variation between temperate and tropical regions, they found that there were different alleles that appeared in the different regions, but the overall genetic variability remained similar in both regions. The diversity of the species and the richness was not significantly different between the two regions (Lehner et al., 2017). This finding can support the fact that even though we found more species and more diversity in the belly button, it was not significantly different compared to the concha. Numerous previous research can support our findings based on the idea that the belly button is a tropical region while the concha is a temperate region.

Looking into the future, we can expand our field of study to other body parts to test and observe if different regions of the body yield more bacterial species. We can compare the habitat of the new sample site to the habitat of the belly button and concha and then make predictions. As we have found, regions that are ‘wet’ tend to have more species, a more abundance of species, and a higher level of diversity compared to ‘dry’ regions. We can use this knowledge to test other regions that could be ‘wet’ or ‘dry’ and observe if this idea remains true. An example of a different ‘wet’ environment could be the armpit while a different ‘dry’ environment could be the forearm. Another expansion of this study could be trying to find every region of the body that has a specific species of bacteria. This would be hard to achieve, but it would allow us to understand how bacteria moves around the outside of the human body. Bacteria of the human body are very diverse and not much previous research has been achieved, so with our experiment we are expanding the field of study and forming a new understanding of the human body bacteria.

Even though the results are consistent with previous research and support our hypothesis, there are still areas of this research that can be improved on. We tried to prevent as many confounding variables as possible by using 20 individuals that are as similar as possible. They were all on the same hockey team, relatively similar in age, and live in the same environment. With that in mind there are still things that vary from individual to individual, such as shower habits, ear cleaning habits, and overall cleanliness. A way to improve this could be to ensure that each individual shower the night before testing and clean their ears the night before as well, with no shower or ear cleaning the day of testing. We don’t know if showering or ear cleaning has a major effect on the present bacteria, but it would allow our samples to be as similar as possible as well as open a new idea for research, the effects of showers on bacteria present on the human body.

All in all, we can conclude that the belly button has a larger abundance of bacterial species and a larger species diversity compared to the concha of the ear. Our finding is consistent with previous research in comparing tropical environments to temperate environments as in our case the human body was a model with the belly button being a tropical environment and the concha being a temperate environment. We have touched on previous studies to try to explain our results and then expanded on ideas for future research as well as provide ways we can improve on our study. Reflecting on our experiment, it was very interesting to see how biogeographic patterns appear all around us without us even noticing.

References

Evans, C. A., W. M. Smith, E. A. Johnston, E. R. Giblett. (1950), Bacterial flora of the normal human skin. The Journal of Investigative Dermatology, 15(4), 305-324.

Hulcr, J., A. M. Latimer, J. B. Henley, N. R. Rountree, N. Fierer, A. Lucky, M. D. Lowman, R. R. Dunn. (2012). A jungle in there: bacteria in the belly buttons are highly diverse, but predictable. PLOS One, 7(11), e47712.

Lawrence, E. R., and D. J. Fraser. (2020). Latitudinal biodiversity gradients at three levels: linking species richness, population richness and genetic diversity. Global Ecology and Biogeography, 29(5), 770-788.

Lehner, M. S., T. J. de Paula Junior, E. M. Del Ponte, E. S. G. Mizubuti, S. J. Pethybridge. (2017). Independently founded populations of Sclerotinia sclerotiorum from a tropical and temperate region have similar genetic structure. PLOS One, 12(3), e0173915.

Polato, N. R., B. A. Gill, A. A. Shah, K. R. Zamudio. (2018). Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains. Proceedings of the National Academy of Sciences, 115(49), 12471-12476.

Pratolongo, P., N. Leonardi, J. R. Kirby, A. Plater. (2019). Temperate coastal wetlands: morphology, sediment processes, and plant communities. Coastal Wetlands, 2(3), 105-152.

Pyron, R. A. and J. J. Wiens. (2013). Large-scale phylogenetic analyses reveal the causes of high tropical amphibian diversity. Proceedings of the Royal Society B: Biological Sciences, 280(1770), 20131622.

Romer, W. (2013). Hillslope processes in tropical environments. Treatise on Geomorphology, 7(35), 375-381.

Titley, M. A., J. L. Snaddon, E. C. Turner. (2017). Scientific research on animal biodiversity is systematically biased towards vertebrates and temperate region. PLOS One, 12(12), e0189577.

Wiens J. J., C. H. Graham, D. S. Moen, S. A. Smith, T. W. Reeder. (2006). Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. The American Naturalist, 168(5), 579-596.