BatCode: Biodiversity education through portable barcoding workshops

Susan M. Tsang

Introduction

The BatCode Initiative is a series of barcoding workshops that will be brought to Southeast Asia, particularly Indonesia, to provide biodiversity education to non-experts. BatCode aims to improve the long-term success of barcoding initiatives in two ways: 1) by creating incentive for researchers to want to mentor students and invest time in the projects, and 2) focus our research on a specific taxa and region to create a structural framework upon which questions may be posed.

Barcoding initiatives may often have a difficult time of finding mentors who are invested in the project beyond the initial phase, yet students often produce work or preliminary data that may be critical to larger biodiversity studies and should receive recognition for the effort they have put into the project. Barcodes are often only the first step for identifying interesting biodiversity questions and may be utilized as preliminary data for applying for more grant funding, which benefits the researchers. The students may then choose to follow-up on the project and continue to be mentored, which provides them opportunities for continuing on a science track. Having laboratory and analytical skills that use modern tools will also build more in-country capacity for addressing issues such as wildlife crime, as these tools are increasingly becoming an important part of identifying provenance of evidence. Being able to wield these tools and skills successfully will allow students to become more globally competitive and able to contribute to local efforts to use science to address the most pressing challenges of the Anthropocene.

Many students from developing countries have shown an interest in learning about molecular biology, but the connection between genetics, biodiversity, and conservation are often not clearly made. In Indonesia, students who are on the molecular or cell biology track are often not taking courses that pertain to ecology, evolution, or conservation and do not interact in the same study groups as their peers who are ecology or conservation-focused. By offering this workshop as a training course for DNA barcoding, we will bring together dialogue between students from different disciplines of biology and demonstrate the utility of genetic tools to environmental issues. We also encourage our local hosts to help us recruit students who are not from science backgrounds for the workshop so that they may better understand the connection between biodiversity, genetics, and society.

BatCode provides a specific emphasis on Indonesian bats as a way of focusing efforts on an understudied and persecuted taxa. Bats are the second most diverse group of mammals, yet remain some of the most understudied and persecuted animals in the world. The Age of Discovery for bats has not ended yet, with an increasing number of novel species recognized each year from the Paleotropics. With a clearer understanding of the biodiversity native to each region, more accurate assessments of biodiversity and conversation management planning can be made to determine the effect of threats on native biota and ecosystems. However, most of the species described from the Indo-Malayan region often only have morphological or echolocation call data and no genetic data associated with the species. Without this data, unique genetic lineages may not be discovered and conservation decisions cannot be made. This data is particularly important when considering bats, which often look incredibly similar to non-experts.

Workshop activities also aim to build confidence in the students for communicating and discussing science to their peers and local communities, a skill that is not practiced enough in the

lecture-style classrooms of Indonesia. This focus may allow for more complex questions to be asked about biotic assemblages since multiple species may be well represented in the overall sampling. This will allow the team to teach students about development of scientific methods and demonstrate to them how one's results may help build new hypotheses and future experiments. Additionally, BatCode will specifically use a small class size in order to ensure that students receive personalized attention to address their needs and also ensure that every person in the workshop is able to get practical with practical and technical skills. This is often a limiting factor in the current classroom climate in Indonesia due to limited resources and this workshop aims to provide more one-on-one interaction to promote an openness of the student to the teacher that is more similar to collegial relationships at the graduate coursework level.

Objectives

This section details the initially written objectives and degree of achievement of each of these objectives.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
To create a new appreciation for and perception of biodiversity through genetics			Fully achieved	Our workshop indicated an increase in participant appreciation perception of bat biodiversity and general biodiversity concepts through the pre- and post-assessments. The final long-term re-assessments are still being processed, as the gap time between assessments has not finished yet
To teach basic concepts of barcoding, conservation, and practical laboratory skills to at least 100 Indonesian students from 3 sites		Partially achieved		We were only able to hold 2 workshops at 2 sites during this year due to scheduling limitations of the workshop team, and have plans for a third and fourth site in order to fully achieve this goal. In total, 47 students from 4 workshops, along with 12 lecturers, laboratory assistants and other scientific staff participated in this workshop. Assessment data were collected only from students due to scheduling and cultural barriers with assessment of more senior participants.
To generate barcodes for all			Fully achieved	We were able to successfully amplify barcodes for all captured

captured bats				specimens, along with specimens that students brought in
To open a dialogue with students and locals about biodiversity and conservation			Fully achieved	We were able to successfully engage in dialogue regarding biodiversity and conservation, particularly in ways that were not previously taught at the university previously and synthesizing this with wider issues such as disease ecology and wildlife trafficking.
To teach non-experts about bats and their importance to the environment			Fully achieved	With the exception of 3 of the students, experience working with bats was very limited. The link between bats and their importance to the environment was not only very clearly made, but interesting questions from the students were asked as to how this may affect other conservation concerns
To improve the long-term success and impact of barcoding projects by creating networking opportunities and incentives for researchers to participate in BatCode and mentor students		Partially achieved		This can only be partially assessed currently, as the time frame for this objective has not been reached yet. This did create new opportunities for collaboration between students and the lecturers—many of the students have continued to contact the workshop leaders on other scientific projects they are working on, and many brought in data they are working on to ask for help regarding the analyses. This also created new opportunities for international collaboration between the workshop leaders and professors at UI and UNHAS which are new and would approach new questions.

Methods

The two hosting institutions for the workshop were University of Indonesia (UI) in Depok, West Java and University of Hasanuddin (UNHAS) in Makassar, Sulawesi. Participants were not limited to these universities though, as circulars were sent out through various forms of social media and through university faculties to recruit for the workshop. In total, each workshop had 12 student participants (with 1 dropping out last minute due to unforeseen scheduling conflicts), and the hosting departments sent several staff members to also attend, totaling to 15 people per workshop. Recruited participants were asked to send their CV and a short paragraph about their goals, past relevant experiences, and what they hoped to learn from this workshop in order to help gauge level of expertise and English proficiency such that workshop instructors could adjust teaching to student needs. For students with no scientific background, they were instructed to write a paragraph relating to why they were interested in this workshop. Each university held 2 sessions of the workshop, with the first one being for undergraduates only, and the second for graduate students and higher levels of education. This was done to counteract any potential hierarchy-related social issues that can impede a collaborative learning environment where all students feel comfortable asking questions and participating. This method was successful in removing any potential conflict, and also gave opportunities for those further along in their career to meet others who they may work with in the future and ask more in-depth questions that may not be within the purview of knowledge of the undergraduate students.

Each workshop took place over the course of a week: One day for instructors to arrive at the site, one day for set-up and meeting with local hosts, three days for the workshop, one day for post-meeting evaluation and future project assignments, and one day for packing. We permitted students to fill out surveys in Bahasa Indonesian if they felt more comfortable doing so. All course materials were provided as a step-by-step checklist. The class were taught primarily in English, but each section will be taught with an Indonesian team member reiterating the instructions to ensure no confusion due to lack of English fluency. We would also do each step together as a group, giving time for two instructors to help people individually while the leading instructor could continue leading the exercise. With 3 instructors in total for the classroom, this allowed for efficiently helping the students during practical activities.

All project activities can be broken down in four types: lectures, activities, labs, and bioinformatics seminars. For the labs and bioinformatics seminars, students were organized into small groups to work together (generally, teams of 3 people). Students with complementary backgrounds (*e.g.* someone with strong English proficiency with someone who is not as fluent) were placed in the same group to help reach learning objectives.

Lectures:

- Introduction to DNA barcoding – A lecture defining barcoding, with examples of what questions barcoding could be used for addressing in biodiversity research, conservation, wildlife forensics, and more
- Bats of Indonesia – A lecture about the biodiversity and importance of bats to Indonesia's ecosystems, economy, and future
- DNA barcoding protocol overview – A lecture describing the lab protocol to familiarize the students with terminology and provide them a foundation of the rules of working in a lab
- Personal protective equipment, capture protocol overview, and set up demonstration – A lecture on the importance of having personal protective equipment and small discussion to understand the relationship between bats, humans, and pathogens.

Capture protocol will be discussed and instructors will demonstrate how to set up both mist nets and harp traps, and how to choose appropriate sites for setting up traps. Proper care of each of these items will also be discussed.

Activities:

- Practice molecular techniques – Students will practice pipetting and other lab skills that they do not have experience with using water and loading dye in this first section to ensure higher accuracy when doing the actual experiment later.
- Monitor nets and traps – Mistnets and harp traps will be set up with the instructors, with checks every 20 minutes or so (time it takes to walk between each trap). Instructors will show students how to safely remove bats from nets and supervise students removing bats. There will be some down time between checking the nets, which will provide opportunities for the students and instructors to get to know one another better as well. We will hold multiple nights of trapping so that students become more familiar with the techniques and see the bats up close.
- Measurement and identification techniques – Bats will be processed in a central "base camp" site. Students will learn how to measure and record specimen data for each individual captured based on international standards for collections. We will also show students how to use a dichotomous key for identification and what talk about the natural history of each of the bats captured. Standard poses for photographing bats will be demonstrated, as these are the most informative to other researchers who may look at digital photograph vouchers. During this activity, some other locals may also be present and it is a good opportunity for them to learn more about bats along with the students and may be the first time they see these animals up close.
- Bat release – Responsible release protocol for bats will be demonstrated and practiced. Nets and traps should be collected at this time as well. How to responsibly clean up in a field site to prevent any negative environmental effects will also be discussed. This is also a good dialogue to have with both the students and locals, as community stewardship and implementing anti-littering policies are important daily practices that can have a positive environmental impact immediately.

Labs:

- DNA extraction – Students learn how to extract DNA from a piece of tissue from the bat using a standardized lab kit. Here, we use a non-invasive biopsy wing punch as a sample from each of the individuals captured. We encouraged students who had samples from other projects to bring them into the classroom to encourage their interest in this workshop. In the case of a rained out catching day, we were provided available tissue specimens from the host university (though, these were generally not of bats).
- Polymerase chain reaction – Students learn how to target and amplify a specific gene, the barcode COI, using polymerase chain reaction.
- Gel electrophoresis – Students learn how to visualize the amplified gene in an agarose gel and understand why this process separates the amplified DNA
- Sample preparation – Students learn how to clean the samples in preparation for sequencing. An explanation of how sequencing works will also be discussed here.

Bioinformatics:

- Using BOLD System Student Data Portal – Students learn how to use the BOLD database to input their data, search for barcodes, conduct basic distance analyses, and calculate other genetic diversity metrics
- Using DNA Subway – Students learn how to process sequence data through, align sequences, create a tree, interpret phylogenies, and transfer data to GenBank

In addition to the educational activities, participants filled out a pre- and post-assessment in order to evaluate the effectiveness of the workshop. Collection of data for evaluation of an environmental education program is rather rare, especially in Southeast Asia, despite its recognized importance to creating focused curricula tackling current environmental issues. Collecting this information allows the BatCode team to identify which areas of instruction may need to be strengthened before future workshops. Concept maps for determining degree of familiarity with the subjects were originally planned as part of this assessment, but this was removed due to the wide breadth of experiences of participants in a single workshop—the assessment would then become more indicative of a few select individuals and not representative of the whole group. We will continue to monitor participant growth and assess project success by counting products of the workshop in the future (*e.g.*, how many projects arise from the initial barcodes, track student progress after workshop and if learned skills are used again).

Results and Discussion

Here, we report both the educational and scientific results of this workshop, as both pertain to the goals of the BatCode Initiative. Data were only collected from the student participants and not the university staff, for scheduling and cultural reasons pertaining to sensitivities about hierarchy. In total, we had 47 students ranging from the ages of 24 to 43, with multiple representatives from every major island in Indonesia. While the participants did draw heavily from the host institution, 17 participants were from other institutions, agencies, or organizations, each a wholly different one. The backgrounds of the participants were quite variable, with a majority being from universities or NGOs. There were also many representatives who were vets, fisheries management staff, animal rescue center volunteers (who do not necessarily have a science background), and forestry/herbaria staff. Of the non-scientists, there was an architect and a police officer. Only 3 of the participants have prior in-depth experience and interest in bats, though they did not work on genetic aspects of bats.

Figure 1. Photo of one group of students from University of Indonesia Batch 2 with workshop lecturers under one of the mistnets set up on campus.

Figure 2. Students measuring bats in the field.

Students were able to successfully extract DNA, amplify the barcoding gene COI (cytochrome oxidase I), and visualize the DNA using gel electrophoresis. Students were provided with two datasets of bats to work on during the bioinformatics portion of the workshop, one of frugivorous *Pteropus* bats and one of insectivorous *Myotis* bats. Students were tasked with creating a phylogenetic tree using DNA Subway and then interpret what those relationships mean. We completed one set of analyses together as a whole group, and then divided students into pairs to complete the second dataset on their on their own. All students were able to complete this exercise in the classroom, and the post-assessment for this portion is still being conducted. We have maintained a Google Group for all the workshop groups in order to address questions that students may have regarding analyses and anything we have gone over during the workshop.

Figure 3. Students at work on different tasks in the laboratory portion of the workshop.

In total at the West Java site, 9 bats were caught, and identified as *Cynopterus brachyotis*. In one of the males, a batfly was also collected. The genetic data corroborates that the bat is *Cynopterus brachyotis*, though the verification process and quality checks by BOLD leave the data still on hold from submission to the database. The number of individuals sample would represent an order of magnitude increase in the amount of genetic data from wild-caught bats from this part of Indonesia that would be publicly available. The batfly was also successfully sequenced, and identified as the species *Eucampsipoda sundaica*. This is the first genetic data of

this species from Indonesia, and will be made publicly available via the BOLD database, and the data will be replicated in more widely used database, GenBank. In Sulawesi, we were unable to capture any bats, despite multiple nights of sampling effort, and resorted to using fish tissue specimens available from the university for the laboratory activities. The data were successfully captured, and contributed to the research project of one of the students at the university. The fish data are still in the process of being analyzed by the students, and release of that data will be subject to timing decided by the university PI.

In the assessment of learning outcomes, we pooled all participants from both the first and second batches, as there were no statistically significant differences in their assessments due to age or career level. Survey questions were grouped generally into five sections about the participant's level of knowledge of factual information, familiarity with relevant skills, familiarity with biological concepts, level of interest in this subject, and ability to connect the concepts to broader theoretical concerns. In all of these questions (with the exception of one in "Interest," there was a significant increase in post-assessment scores. The one exception within "Interest" may be due to the self-selecting nature of the workshop—participants who bothered to sign up for it are likely already somewhat interested in learning about the subject matter presented in this workshop. Age was not predictive of skill, experience, nor knowledge of facts, and points to the general usefulness of practical workshops tied to larger theoretical concerns to the Indonesian scientific community. Surprisingly, we did not target English language learning specifically as part of this workshop, but post-assessments demonstrated a vast improvement in English proficiency by participants. 90% of the participants filled out post-assessments in English, as compared to the 20% for the pre-assessments. Our emphasis on shared learning also increased the willingness for participants to engage in their own outreach efforts in their own departments or institutions/organizations, some of which they did not feel comfortable doing prior due to lack of feeling of being an "expert."

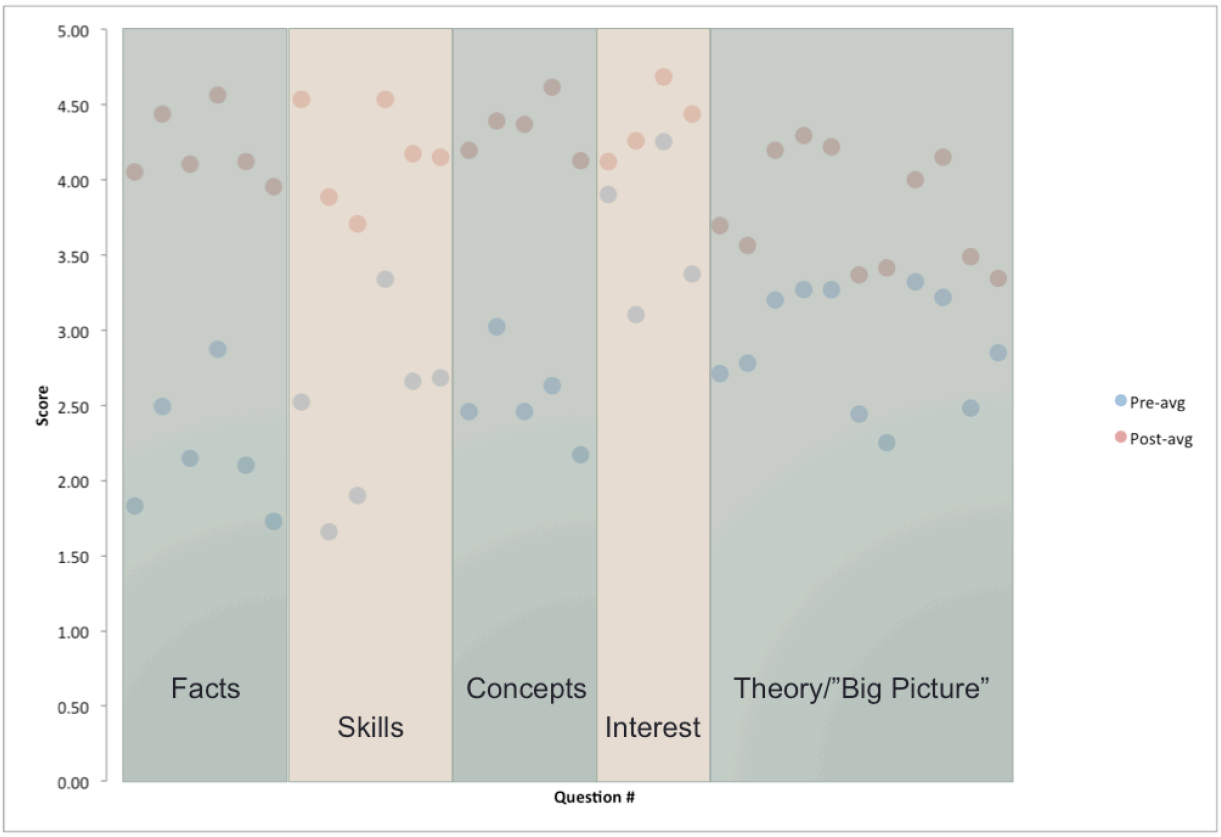

Figure 4. Comparison of average scores for pre- and post-assessment questions for all participants.

Conclusion and Future Directions

The third and fourth workshops in order to reach the 100 student goal initially proposed will be conducted Summer 2017. The targeted sites that have expressed interest in engaging in this work are in Bali and West Kalimantan, and the logistics of finding a suitable local host are currently being negotiated. We aim to also take the findings from the first round of workshops and create larger research programs with students and local researchers interested in these questions and making them into viable options for thesis projects for undergraduates or Master's students. We aim to also produce a set of useful guides for bioinformatics questions that often arise from Indonesian students, as that is one of the largest gaps in expertise in Indonesia. Our workshop has helped indicate where more explanation is needed because there are no subject matter experts easily available to students, and we will widely disseminate these materials, which will be in Bahasa Indonesian, after its production. We will also track outreach efforts made by participants from this workshop to determine its impact on non-experts.