International Journal of Research and Review
Vol. 10; Issue: 4; April 2023
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Development of DNA Barcoding Student Worksheet for Biology Undergraduate Students in Invertebrate Taxonomy Course

Eunike Manurung[1], DS Diningrat[2], Binari Manurung[2]

[1]Biology Education, Undergraduate student, Universitas Negeri Medan,
[2]Lecturer of Biology Education, Undergraduate, Universitas Negeri Medan,
Jln Willem Iskandar Pasar V Medan Estate - 20221

Corresponding Author: Eunike Manurung

DOI: https://doi.org/10.52403/ijrr.20230461

ABSTRACT

DNA barcodes have revolutionized the insect taxonomy field and made the learning process of taxonomy more accessible to everyone. This study aims to create student worksheets on DNA barcoding to improve student achievement in the learning process of insect taxonomy. The research was conducted from September to December 2022 using the 4D model ((define, design, develop, and disseminate). The study involved experts in material/content and learning approach, subject lecturers, and students. The developed student worksheet product underwent validation, assessment, and response from material/content experts (95.83%), learning approach experts (95%), subject lecturers (83.33%), small group test (94.66%), and big group test (94.90%), which indicated that it was very feasible to use. Before implementation, the results of paired t-test between the control and experiment pretest results were t count = 1.76 (p = 0.08); df =31), meaning there was no significant difference between the control and experiment pretest results. However, after implementing the student worksheets that have developed, the paired t-test between the control and experiment posttest results was t count = 6.67 (p = 0.00; df = 31), meaning there was a significant difference in learning outcome between the control and experiment posttest results. Moreover, the N-gain value obtained in the experiment class was 0.9107 means "high," indicating that applying student worksheets are more effective than just using a collection of books in increasing student achievement.

Keywords: Student Worksheet, DNA Barcoding, Insect, Research & Development (R&D)

INTRODUCTION

In the world of biology, taxonomy is a branch that discusses the classification of living things. The aim is to identify and name the various species present and establish the relationships between them. Invertebrate taxonomy is an essential field of study because it helps to organize and understand biodiversity, especially invertebrate animals. Unfortunately, this branch of biology needs to be addressed and served. Only a few students are interested in studying taxonomy because they think taxonomy is a tiresome and unnecessary science. This is shown by the diminishing number of students who choose to write articles in taxonomy [1]. In fact, the magnitude of the biodiversity of an ecosystem will only be known if a broader taxonomic study is carried out on that ecosystem.

Despite their importance in maintaining ecosystem health and function, insects are often overlooked or under-studied compared to larger, more charismatic species such as mammals or birds. This is especially concerning given that many insect species

are facing unprecedented declines due to habitat loss, climate change, and other anthropogenic factors [2].

As one of the most diverse groups of invertebrate organisms, insects require special attention to their classification. The traditional approach to studying insect taxonomy is primarily based on morphological features. However, this approach has limitations, as some species may share morphological similarities despite being genetically distinct. This leads to misidentification and inaccurate species classification. Furthermore, morphological identification is subjective and requires specialized knowledge and extensive training, making it difficult to scale up studies.

In contrast, DNA barcoding relies on a short region of the mitochondrial cytochrome c oxidase I (COI) gene, which is highly conserved within species but differs between them. This method provides a reproducible identification system and overcomes the limitations of morphological identification. As a result, DNA barcoding effectively identifies several cryptic insect species, including the white leafhopper, leafhopper, and fruit fly [3,4,5,6]. Another thing sequencing technologies have made it possible to generate molecular data on a large scale, thereby quickly providing a comprehensive view of the phylogenetic relationships between taxa. Furthermore, integrating molecular data with ecological and morphological information can help reveal species' evolutionary history and ecological adaptations. Thus, DNA Barcoding has significant implications in insect taxonomy research and has the potential to contribute to the sustainable management of global biodiversity.

Therefore, now we need the help of DNA Barcoding which is carried out based on a genetic/molecular approach. Although DNA barcoding does not replace comprehensive taxonomic analysis, it can be used as a starting point, as it provides an initial signal of population analysis divergence and facilitates comparative studies of population diversity across species. Likewise, DNA barcoding can complement traditional taxonomic methods by providing a standardized framework for species identification. This allows the integration of molecular data into the revised taxonomy, thereby increasing the accuracy of species identification. In addition, if taught to students, this lab activity provides a variety of situations to introduce the student to the analysis of DNA sequences and the use of DNA databases [7,8]. When actively participating and learning about the introduction and application of DNA Barcoding to identify insects, they can begin to appreciate/think critically about the importance of studying taxonomy through a molecular approach. This means that through DNA barcoding, students not only remember concepts but how they understand scientific concepts and their applications in life.

However, learning about DNA Barcoding takes work. The hope of achieving DNA Barcoding competency for Biology students at Medan State University has been contained in the semester syllabus/learning plan. However, based on interviews and observations conducted with biology students in 2019 and 2020 who have completed invertebrate taxonomy showed that only a few students know the application of the molecular approach to invertebrate taxonomy. This is because they only use conventional techniques when carrying out practicums. In addition, students feel disturbed by learning that is only based on textbooks/presentations; It is necessary to add teaching materials in the form of practicum worksheets to make learning more meaningful and provide special skills.

To overcome this problem, this research aims to make DNA Barcoding worksheets to improve student achievement in the learning process of insect taxonomy. Student worksheets development is significantly needed in education to gain science process skills [9]. The use of worksheets in appropriate situations can

increase mastery of concepts [10]. As a learning resource, student worksheets are guidelines for students to use in research activities and problem-solving. In addition, it can be a guide for training the development of cognitive aspects through practical guides or demonstrations [11]. Student worksheets can activate students in the learning process, support limited media, increase student motivation and curiosity, help students solve problems, and foster self-confidence [12]. Students are expected to have skills/competencies in understanding molecular approaches in taxonomy by using student worksheets on DNA Barcoding in insects. This is because, currently, the community expects universities to graduate students who have 21st-century skills and must be ready to be part of a changing era [13].

The point is that the entire function of student worksheet is to make it easier for the lecturer to present an exciting learning process so that students can understand the lesson. Therefore, making DNA Barcoding student worksheet improves student achievement in the learning process of insect taxonomy. Therefore, finding out the effectiveness of student worksheets to improve student achievement in the learning process of insect taxonomy is very necessary.

RESEARCH METHOD

This research was conducted at the Molecular Laboratory of the Department of Biology, Medan State University, located at Jalan Willem Iskandar, Estate Pasar V Medan, Percut Sei Tuan, Deli Serdang (near the border of Medan City). This research was held from September to December 2022. The subjects in this study were content/material experts, learning experts, and one subject lecturer with students taking the Invertebrate Taxonomy course, namely biology student class (PSB) 22 A (32 people) as the control class and PSB 22 D (32 people) as the experimental class. The object of this study was the feasibility of student worksheets with DNA Barcoding on the topic of Insects which were responded to by the expert validation team, subject lecturer, and students. The type of research is research and development (R&D), with 4D development as a research design in this study.

The first crucial step is the define/initial stage which is carried out by analyzing observation, interviews, and questionnaires to lecturers and students so that problems and solutions are found that follow the existing learning requirements in studying the molecular approach to invertebrate taxonomy. The second step in this research is the design stage. In this step, the researcher prepares the competency achievements and indicators that must be fulfilled based on the previous first step. The researcher also designed worksheet products that will be used, starting from the cover with a combination of orange and blue colors accompanied by pictures of fruit flies (*Bactrocera*) with the title Barcoding insect DNA, making instructions for use, making concept maps, making theories about the basis for the introduction of DNA Barcoding (starting from the definition, source of barcodes, benefits, and work steps), provides links and barcodes regarding the video of DNA Barcoding steps to the MEGA XI application software, creates three activity sheets containing work steps and questions, as well as a bibliography. Besides that, the researcher also designed an instrument to determine the product's feasibility starting from validation sheets, response questionnaire sheets, and learning achievement tests (pretest and posttest questions).

In the third step, the development stage, the student worksheet products that have been worked on are then validated by material/content experts and lesson approach experts and are responded to by the subject lecturer and students on the scale of small group tests and big group tests so that student worksheet products are not misinformation and conform with the intended target [14, 15]. By using a questionnaire, we can perform calculations

using the feasibility formula and interpret it based on the scale table and interpretation that was made previously. In the fourth step, the disseminate stage, after the pretest, the product is implemented into learning in the experimental class. When finished, a posttest was carried out using the same questions. The results of the pretest and posttest of the experimental and control classes were then analyzed using a paired t-test to find out how far the differences between the pretest and posttest scores of the two classes were and continued with the N-gain test to determine the effectiveness of student worksheets in improving student achievement [16, 17]. All existing data has been analyzed using SPPS v.25 software.

RESULT

This research on developing student worksheets with DNA Barcoding on Insert topic uses a Research and Development (R&D) approach that refers to the 4-D models. The results of this study explain the process based on the research methods implemented as follows: Define stage: In this step, analysis is carried out both in terms of objectives and limitations to determine the product requirements to be developed. From observations, interviews, and questionnaires, it is necessary to develop insect DNA barcoding worksheets to increase understanding and even skills regarding the molecular approach. After forming a concept map based on The Course Learning Outcomes (CPMK) in the Invertebrate Taxonomy syllabus, it was found that students must at least understand the stages of implementing DNA Barcoding, starting from PCR amplification, blast analysis with MEGA and NCBI, and analysis of molecular data use MEGA to find the phylogeny.

Design stage: At this stage, a student worksheet for insect DNA barcoding is produced with eight components, namely a guidebook, competency achievement, and indicators, a concept map, theory information, supporting information, the activity sheet, exercises, and evaluation. At this stage, the researcher also produced validation instruments for material/concept experts (12 questions), validation instruments for learning approach experts (10 questions), questionnaire sheets for subject lecturers (12 questions), and questionnaire sheets for small groups (12 questions) and big group (17 questions) consisting of 4 answer choices namely 4 (very worthy), 3 (worthy), 2 (less worthy), and 1 (not worthy). The pretest and posttest questions consist of the same questions designed in the form of essay questions to determine the increase in learning outcomes after students participate in learning.

Development: At this stage, the results of expert validity tests, subject lecturer assessments, and student responses are produced (Table 1). Based on the validation results, which were between 83.33% - 94.90%, it can be concluded that the insect DNA barcoding worksheets that have been developed are feasible to be implemented in teaching and learning activities, and their effectiveness will be known.

Table 1. Results of validity

Expert validation	Mean Score (%)	Feasibility
Material/ content expert	95.83%	Very Worthy
Learning approach expert	95%	Very Worthy
Subject Lecturer	83.33%	Very Worthy
Small group test (students)	94.66%	Very Worthy
Big group test (students)	94.90%	Very Worthy

Before entering the teaching and learning activities, a pretest was conducted in the control and experimental classes to determine the initial results of students' understanding of DNA Barcoding. Then the control class carried out learning using books originating from a collection of several books, while the experimental class carried out learning using student worksheet products that were developed. After the teaching and learning activities were completed, a posttest was conducted in the control and experimental classes to determine whether there were differences in results. The results of the pretest and posttest learning outcomes in the control and experimental classes based on descriptive statistics are shown in Table 2.

Table 2. Learning Outcome Results in Pretest and Posttest

	Pretest		Posttest	
Variables	**Control**	**Experiment**	**Control**	**Experiment**
Mean	11.68	13.34	73.06	92.25
N	32	32	32	32
Std. Deviation	4.70	5.51	14.60	5.96

Based on the results of the pretest and posttest in both classes (Table 2), it was found that the experimental class produced a high increase from pretest to posttest (13.34-92.25) compared to the control class (11.68 - 73.06). Nevertheless, it does not end there; normality and homogeneity tests are carried out to obtain more significant and accurate data using a paired t-test [16]. The results of the normality and homogeneity tests by the two classes are presented in Table 3 and Table 4.

Table 3. Normality Test Results in Pretest and Posttest

Class	**Kolmogorov-Smirnov**		
	Statistic	**df**	**Sig.**
Control Pretest	.172	32	.160
Control Posttest	.186	32	.070
Experiment Pretest	.101	32	.200
Experiment Posstest	.168	32	.220

Table 4. Homogeneity Test in Pretest and Posttest

	Levene Statistic	**df 1**	**df 2**	**Sig.**
Based on Mean	1.38	1	62	.24

The normality results based on Kolmogorov-Smirnov test showed that the pretest and posttest values in both classes were normal (P = 0.70 – 0.22: P. > 0.05) (Table 3). The homogeneity test results also showed that the data was homogeneous (P = 0.224: P. > 0.05) (Table 4). That was, the data can be used to perform a paired t-test. The results of the paired t-test in control and experimental classes are shown in Table 5.

Based on the pretest that had been done in control and experimental class people who had been tested on SPSS version 25, it was found that the results of the t-test between the control and experimental pretest results were the acquisition of a significant value of were t count = 1.76 (p = 0.08); df =31) (Table 5) which means that based on the acceptance criteria of research concludes "there is no significant difference between the control pretest results and the experimental pretest results at insect DNA Barcoding topic."

However, after implementing the student worksheet product, the results of the t-test between the control and experiment posttest results from the acquisition of a significant value of t count = 6.67 (p = 0.00; df = 31) (Table 5), which means 0.00 ≤ 0.05, so based on the acceptance criteria of research conclusions, "there is a significant difference between the control posttest results and the experimental posttest results at insect DNA Barcoding topic."

Table 5. Paired Samples Test Results in Pretest and Posttest

		Paired Differences							
		Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**		**t**	**df**	**Sig. (2-tailed)**
					Lower	**Upper**			
Pair 1	Control Pretest - Experiment Pretest	-1.65	5.30	.93	-3.56	.25	-1.76	31	.087
Pair 2	Control Posttest - Experiment Posttest	-19.18	16.25	2.87	-25.04	-13.32	-6.67	31	.000

The results of these two pairs (Table 5) showed that before the insect DNA Barcoding learning activity was carried out, the learning outcomes of the two classes were in the same position. However, after conducting the posttest, it was found that students who used insect DNA barcoding worksheets had an average score higher than students who did not use insect DNA barcoding worksheets. So that the insect DNA Barcoding worksheet product can be used to increase student achievement. To justify this statement, the N-Gain test is also carried out [17]. The N-gain results based on pretest and posttest data in the control and experimental classes are shown in Table 6.

Table 6. N-Gain score results

Variables	**Class**	
	Control class	**Experiment class**
Minimum	0.28	0.67
Maximum	0.88	1.00
N- Gain Score	0.69	0.91
Category	Medium	High

From the results above, it is known that the average N-gain score in the experimental class that uses insect DNA Barcoding worksheet products is 0.91 or 91% with the interpretation of "High," where the minimum value is 0.67 or 67%, and the maximum is 1.00 or 100 %. Conversely, the average N-gain score in the control class that does not use a student worksheet is 0.69 or 69% with the interpretation of "Medium," where the minimum value is 0.28 or 28%, and the maximum is 0.69 or 69%.

DISCUSSION

Various stages are needed to produce a student worksheet product that is useful and in accordance with the objectives. At the define stage, all potential and existing problems have been exported because brain activity and physical activity contribute to the many factors that influence student learning outcomes [18]. At the design stage, researchers must be able to pay attention to things that seem small, such as the cover and even the structure of the content. This is because a well-designed cover can increase the student's motivation, interest, and overall learning experience [19], and the structure of the content influences students' understanding of the subject matter [20].

At the development stage, the DNA Barcoding student worksheet products were tested by various parties. The material/ content expert validates material presenting feasibility aspects; the learning approach expert validates content feasibility aspects and components of the scientific approach; the subject lecturer responds to content aspects and language aspects, while students respond to student worksheet display aspects, language aspects, and graphical aspects (especially for the big group test). The suggestions and improvements from the validator were carried out in order to obtain an appropriate student worksheet product. It is essential to seek validation from material experts to ensure accuracy and effectiveness based on the intended audience so that misconceptions and the use of information can be avoided. On the other hand, validation by learning approach experts is essential to ensure that the contents of student worksheets are well-designed and presented to maximize student learning outcomes. In addition, seeking feedback from subject lecturers can help ensure student worksheets meet course goals and objectives [21]. Meanwhile, from student feedback, we can find their challenges in using student worksheets. For this reason, small-group tests and big-group tests were carried out [22] as the presentation of high, medium, and low-level students.

At the disseminate stage, after the student worksheet products were implemented, the paired t-test and N-gain test results were obtained from the pretest and posttest values of the experimental and control classes. The results showed that applying student worksheets with DNA Barcoding for Insect topics is more effective than using a collection of several books. From these results, we understand that the student worksheets that have been developed can guide students through problem-solving exercises and encourage active learning through critical thinking, analysis, and evaluation of molecular biology concepts. A study conducted by Solihah & Aditya (2020) found that the learning achievement of students who studied with guided inquiry-based student worksheets and the learning achievement of students who studied with modules had a significant average difference, which was the learning achievement of students who studied with student worksheet based guided inquiry has a higher average than those who study with modules [23].

This study shows that worksheets encourage student engagement in the learning process, which leads to improved learning outcomes. Student worksheets provide direction and guidance for learning so that students focus on the flow of material presented [24]. The structure of the student worksheet consists of conceptual knowledge, preparation of equipment, and scientific steps. Because the students not only accumulate new knowledge but also develop their

understanding of scientific processes and methods, enabling them to explore the world around them. So, the use of student worksheets is expected to increase student knowledge retention [25].

In addition, worksheets with an inquiry approach provide a platform for students to ask questions and find solutions to concepts they do not understand. This interaction encourages active learning and encourages students to take responsibility for their learning outcomes. A Sayuti (2019) study found that student worksheets provide opportunities to connect new information with students in cognitive, psychomotor, and practical structures [26].

Therefore, using student worksheets in subjects related to the molecular approach positively impacts student learning outcomes. These worksheets increase student engagement, encourage critical thinking, help students retain knowledge, and improve their attitude toward the discussed content. As such, student worksheets should be included when banning DNA barcoding as an effective tool to encourage active learning and improve student learning outcomes.

CONCLUSION

Based on the results of the research, analysis, and discussion that has been presented, it can be concluded that the feasibility of the insect DNA barcoding student worksheet product that has been developed was very worthy/ feasible according to the material/content and learning approach experts' validation, subject lecturer assessment and student responses in the small group and big group test. Therefore, the insect DNA Barcoding student worksheet product that has been developed is effective for increasing student achievement and is included in the "High" category.

Declaration by Authors

Acknowledgement: None

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Eunike Manurung, DS Diningrat, Binari Manurung. Development of DNA barcoding student worksheet for biology undergraduate students in invertebrate taxonomy course. *International Journal of Research and Review*. 2023; 10(4): 498-505. DOI: *https://doi.org/10.52403/ijrr.20230461*

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