UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO:

Design of novel ways to reduce the populations of black flies in the valley of Urcuqui

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniero Biomédico

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Dedication

This thesis is dedicated:

To my mother Mara Emilia Bello who motivated me to study at this prestigious university, Yachay Tech, and unconditionally supported me to finish my studies. Thank you for inspiring me to be a better person and also to be a great professional.

Also, to all the people involved in my academic life, including my tutor, Francisco Alvarez Ph.D, co-tutor, Diego Almeida Ph.D. Si Amar Dohoumane Ph.D., Frank Alexis Ph.D., Hortensia Rodríguez Ph.D., Sandra Hidalgo Ph.D., they show me the passion and dedication to transmit their knowledge.

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Resumen

Los simúlidos son moscas negras que afectan a la salud y el bienestar de muchos mamíferos, incluidos los humanos. Las hembras de estos insectos necesitan alimentarse de sangre para poner huevos y para la maduración de estos. En consecuencia, varias especies de estas moscas son transmisores de patógenos que causan enfermedades. En particular, la presencia excesiva de simúlidos en el valle de Urcuquí de la provincia de Imbabura, son causa de gran malestar tanto para los propios residentes como para los visitantes. También son causa de perjuicio económico y de salud para sus animales, tanto domésticos como los dedicados a la agricultura y la ganadería. En este trabajo hacemos una propuesta de estudio desde muy diferentes perspectivas y proponiendo varias soluciones al problema. La propuesta abarca la identificación de las especies de simúlidos, la búsqueda de atrayentes o repelentes, la identificación de individuos resistentes a las mordeduras de los insectos y la creación de estrategias y dispositivos para su eliminación de lugares concretos en el valle de Urcuquí.

Palabras Clave: simúlidos, mosca negra, patógeno, dispositivo.

ABSTRACT

Simuliids are black flies that affect the health and well-being of many mammals, including humans. The females of these insects need to feed on blood to lay eggs and for their maturation. Consequently, several species of these flies are transmitters of pathogens that cause disease. In particular, the excessive presence of simuliidae in the Urcuqui valley of the province of Imbabura are a cause of great discomfort for both residents and visitors. They are also a cause considerable economic losses and poor health in animals, both wild and domestic, as well as people, especially those dedicated to agriculture and livestock. In this work, we propose a comprehensive study of simuliids from very different perspectives and propose various solutions to the problem. The proposal includes the identification of simuliid species, the search for attractants or repellents, the identification of individuals resistant to insect bites and the creation of strategies and devices for their elimination from specific places in the Urcuqui valley.

Key Words: simuliids, black fly, pathogen, device

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1 Introduction

Black flies are the common name given to blood-sucking insects of the family Simuliidae. They belong to the order Diptera, which includes 2,072 species worldwide and 12 genera and around 359 species described in the Neotropical region (1). They are small (2 - 5 mm long) flies of dark color (giving them the common name of black flies), short legs and wide wings. Their bite can produce allergic reactions in their victims, and some species are known vectors of diseases (2). Their life cycle consists of a larval phase in an aquatic environment and an adult phase in a terrestrial or aerial environment. Simuliidae have evolved in such a way that they now coexist with humans and domestic animals.

Both sexes of these species feed on nectar from plants or flowers. In addition, these Diptera are hematophages, whereby it is the females that ingest blood. This blood is used for energy storage in the eggs. The flies bite during the day, although some species prefer to feed at dawn or dusk. Females oviposit large masses of eggs in streams, and the larvae emerge within a few days. The larvae avoid being dragged by the water currents by a silk thread secreted from their mouth, until they find a support substrate such as rocks, leaves, branches, etc. (3). The life expectancy of these species is not entirely known, but it is believed to vary between two to three months in temperate zones. It should be noted the ability of this specie to adapt to some environment (3).

On the other hand, black flies have become a serious problem as vectors of infectious agents for humans and animals. For example, they are known to transmit the parasitic nematode *Onchocerca volvulus*, causing agent of the “river blind disease” or onchocerciasis in parts of Equatorial Africa and mountainous regions of northern South America and Central America. Besides the risk of acquiring such infection, the painful bite from female flies is a problem itself. Being bitten by flies in rest areas such as parks, bus stops, among other places, is a cause of common irritation that has an important economic impact since it affects the normal development of various activities, such as of tourism, agricultural tasks and animal production. Hence, this results in an economic burden for the health and sanitation services (2) of the communities affected.

The present study is centered on Urcuqui, a small town in the Imbabura province of Ecuador, with a natural beauty with the biodiversity proper of dry-forest ecosystems and a network irrigation canals. In the valley of Urcuqui, a landscape of agricultural fields and pastures predominates. In addition, the beauty of the location makes it attractive for national and foreign tourists. The yet unfinished construction of Yachay Tech University in the Valley of Urcuqui is a great attraction for tourists as well as researchers in this center for innovation unique to the northern provinces of Ecuador. Unfortunately, black flies have been annoying residents that attack both humans and domestic animals in the area. The presence of the flies can thus result in great economic losses for the residents, because the presence of the black flies affects the amount of visitors, increase spending of agricultural products, and other every day economic activities in the valleys. Consequently, it is necessary to generate novel solutions to effectively control the population of the black flies in the valleys described above.

In this work, a number of proposals for research and innovation are put forward that could be developed and implemented in the near future for the effective control of the local black fly species, aiming at an integrated management between biology and engineering as the best alternative to control this pest.

2 Theoretical framework

2.1 Biology and Ecology

2.1.1 Life cycle of the black fly

The Simuliidae constitute one of the most relevant Diptera families in fluvial environment, where this holometabolic species develops; for this reason, these species are of great biological and ecological interest since it presents morphological adaptations and a peculiar behavior (1). Simuliids are known by different names such as: blood-sucking flies, carmelites, drunkards, gnats, mbarigui, jerjeles, paquitas or petros (5).

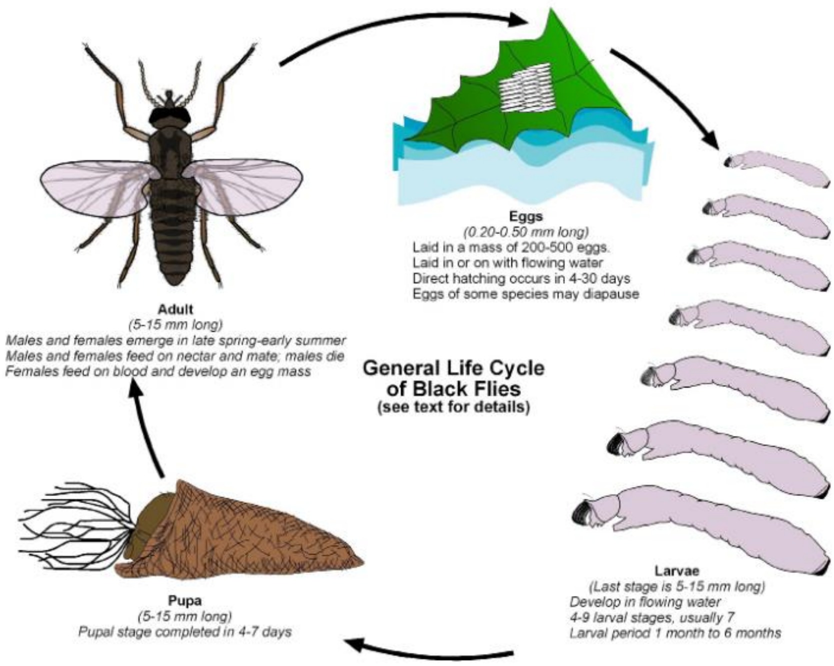

Figure 1. *General life cycle of black flies. (Illustration by: Scott Charlesworth, Purdue University, based in part on Peterson, B.V., IN: Manual of Nearctic Diptera, Vol.1*

This black fly goes through complete metamorphosis. In addition, its life cycle is linked to aquatic habitats and includes the four stages described in Table 1 such as eggs, larvae, pupae and adults. In its first stage, a single female can deposit between 200 and 300 eggs (6); these are deposited on plants or rocks near river sources. Later, after 1 to 4 days, they hatch, so the larvae are located in an optimal place for their development. In addition, the larva must go from 6 to 9 stages of instars that require between 2 weeks to 20 days (7). The immobile pupal period begins once the larva has woven a silky cocoon. In this period the fly does not feed and protected from the water. Finally, metamorphosis occurs. For this process the developing fly requires large amounts of oxygen from the water until it forms a bubble inside the cocoon. In this way, individuals rise to the surface and must pose for a time to dry and harden the exoskeleton and cuticle. Thus, they reach their final size, whereby males are smaller than females. The fly's body has an elongated abdomen with ten segments, a pair of wings, a pair of halters, and three pairs of legs. Its wings measure between 1.4 to 6 mm and its body reaches 1.2 to 6 mm (7, 8).

Due to the capacity of simuliids to fly, they can reach great distances (2). A study in Guatemala determined that they reach an average flight distance of 7.5 miles (12 km), although species of flies were found in the ears of the horses, which means that they can also travel on horses and reach even greater distances (9). Adults can live from 2 weeks to almost 3 months. In addition, the longevity of the species depends on several factors, such as air temperature, place of residence, season of the year and the presence of food.

***Table 1**. Development times of each stage of the simuliids (3)*

State	Duration (Days)
Egg	1-4
Larvae	1-20
Pupa	14-20
Adult	60

2.2 Taxonomy

So far, there about 2,141 species and 26 genera of Simuliidae have been described (4). The most relevant genus for human and animal health is Simulium (Castex, 2001). León and Wygodzinsky in 1953 were the first people to carry out simuliid studies in Ecuador (10). Furthermore, in the same year the species *S. escomeli* was described in the province of Imbabura with material from Lake San Pablo in Otavalo (11). The first clinical, parasitological and epidemiological studies were carried out between 1979 and 1985 (12). For example, one species of *S. pternaspatha* and 13 species of the genus *Gigantodax* were described (12, 13). Subsequently, two new species were described and four unknown species for Ecuador were cited (14). Until now, 25 species of the genus *Simulium* and 14 species of the genus *Gigantodax* have been cited in Ecuador. However, the samples were not collected in all parts of the country and not for all the periods of the year. Therefore, the description of this family of black flies is believed to be incomplete.

2.3 Diet and damage

Something that has to be highlighted about the Simuliidae family is that, thanks to their morphological characteristics and adaptations to different habitats, several generations of a same species can develop in the same year. This greatly increases their numbers within their niches, which represents a problem for the human inhabitants of the area. The adult individuals of this fly are gregarious, forming swarms. Both females and males require sugary vegetable juices to feed. These provide them with energy for their vital activity, but females also need blood meals for egg development, as mentioned above.

Simuliids are telmophags, since they have serrated jaws with which they rip open the skin of their victims, producing a small lesion on the skin. During the bite, they inoculate a saliva that has anesthetic, antithrombotic, vasodilator and anticoagulant substances that hide pain, while preventing blood clotting and contributing to increased blood flow in the area (15). Therefore, this saliva is responsible for the bite going unnoticed by the victim. The bite becomes painful and/or itchy after a few seconds to minutes, depending on the reaction of each individual person (Fig. 2). In addition, the bite causes inflammation, itching, bleeding, edema, local pain (16), and in some cases medical attention is required. Some people react to the bite of a black fly with a set of symptoms known as "black fly fever." These include headache, fever, nausea, and swollen lymph nodes (17).

a. b.

Figure 2. *Victims of various black fly bites: a. legs of a young man (18). b. dog's belly (19)*

The damage caused by Simuliidae can be both direct and indirect. On the one hand, the direct damages are the bites of these flies, which cause unique diseases such as simulitoxicosis. In addition, this species of fly can transmit bacteria, viruses, protozoa and nematodes to humans and animals, resulting in diseases such as onchocerciasis (16) and leucocytozoonosis (20). However, the full diversity of diseases that are transmitted by black flies has not yet been thoroughly studied.

On the other hand, the indirect damages are losses in the livestock sector. For example, in the USA it is estimated that more than $730 million are lost each year due to some species of fly, including the reduction of 1 to 5% of milk due to stress from the flies annoyance (21). According to the Ministry of Livestock and Agriculture (MAG) in our country one of the main causes of diseases in bovine species is transmission by flies.

As an example, one of the diseases transmitted by simuliids is onchocerciasis, which is caused by the phylatic nematode *Onchocerca volvulus*. Native to Africa, this nematode is now also present in Asia, Central America and South America. According to the WHO, humans are the main host of this pathological parasite which can be found in 31 countries. This parasite has an incubation period of 8 months to 2 years and its pathogenesis is due to the microfilariae, especially the dead ones, which move through the subcutaneous tissues generating inflammatory responses. The symptoms it causes are severe skin and eye lesions, nodules or onchocercoma, lymphedema, partial blindness and that can become permanent (22). Since 2004, no patients with onchocerciasis have been detected in Ecuador, thanks to the intervention of the Pan American Health Organization (PAHO) and the Onchocerciasis Elimination Program for the Americas (OEPA). They declared the disease extirpated, and the surveillance stage began until 2012 to obtain the elimination certification. This success resulted from the administration of a drug known as Ivermectin to the population at risk every six months (23).

Another pathogen transmitted by simuliids species is *Mansonella ozzardipor*, which causes mansonellosis or Ozzard's filariasis. This condition affects the inhabitants of the jungle areas of the American continent (24). Symptoms caused by this pathogen are lymphadenitis, fever, and headache (25). Simuliids can also cause leucocytozoonosis, found mainly in the eastern region of Ecuador, where simuliids transmit *Gomphostilbia* among birds through their bite. Finally, there are other conditions related to simuliids such as myxomatosis (26), Venezuelan equine encephalitis (27) or the vesicular stomatitis virus.

3 Simuliidae as Pests

Many species of Simuliids are a worldwide pest of humans and livestock (28), and in Ecuador has been since 1980, when according to the Ministry of Public Health black flies began to be a serious health problem with the discover of onchocerciasis was registered in the country (29). This led the State to pay attention to this problem, generating control projects for these species. Although nowadays a form of progressive control of it has not been proposed.

3.1 Control

Pest control in Ecuador is a complicated task since the parties involved (government agencies and interested citizens) don't have a plan outlined based on sustainability. To our knowledge, the control of the simuliids is never considered by any region in Ecuador. In countries where the control takes place, the larval stage is usually the main target, due to being spatially delimited. Otherwise, when they are adults, as mentioned previously, flies cover larger, more diffuse areas (30) and the control becomes more difficult. There are several ways to control the population of this kind of pest, such as environmental management, chemical control, biological control, ethological control and cultural action.

3.1.1 Environmental management

The management of the environment, which consists of the elimination of the substrates for the development of the larvae. Here, the canals and the vegetation of the ditches are cleaned to avoid providing habitat for the simuliids. Additionally, the channels of the water tributaries can be artificially manipulated, in order to hinder the development of the developmental stages of this insect and facilitate its predation (31).

3.1.2 Chemical control

In this type of control, the use of insecticides is allowed, as are larvicides and adulticides. For example, the use of the organochlorine DDT in the 1950s was effective, although it was banned in almost all countries due to its adverse effects on organisms (including humans) and thus damaging aquatic ecosystems. Products such as Temephos and Methoxychlor were then used for a long time (32). This gave rise to the appearance of resistance (30). Meanwhile, due to these adverse effects of pesticides, the use of products of bacterial origin has been gaining ground.

3.1.3 Biological control

The most recommended control system is biological control. This control is mostly used on larvae through the spores of the bacterium *Bacillus thuringiensis* serotype *israelensis* (Bti) (33). This bacterium is applied in the places with the highest density of the larvae. The action of this bioinsecticide involves the intake of the bacteria by the spiracle of the insect. This causes an osmotic imbalance, which breaks down the wall of the victim's intestine, causing death from septicemia. Additionally, Bti is selective, in addition to attacking simuliids it also attacks larvae of culicids and some chironomids. There are also larval and adult predators of simuliids that can reduce their population (34). Additionally there are entomopathogens such as viruses, fungi, protozoa and nematodes that act on these insects.

3.1.4 Ethological Control

This type of control is done through the use of traps with food, visual or sexual attractants. In this type of control, the insect is captured for control analysis and monitoring of the population, which allows for evaluation of the population status.

3.1.5 Cultural control

Finally, cultural control is the last pillar of integrated pest control, which encompasses information, communication and education concepts. This action is carried out by raising public awareness through information brochures. These brochures contain information on how to prevent and act effectively against a black fly bite.

4 Problem Statement

The excessive presence of Simuliidae in the valley of Urcuquí is a cause of great discomfort both for the residents themselves and for the visitors. They are also of great economic and health concern. So far, large-scale solutions to the problem have not been developed. In Ecuador the usual control of similar pests implies a great effort at an economic level and causes a notable environmental impact, due to the high number of chemical products used. In addition, the lack of studies of these flies in the country has prevented the generation of effective solutions by universities and other research centers. Due to all these reasons, the present work proposes to analyze this problem from different, novel points of view. First, I suggest the taxonomic identification of the species present in this particular area of the province of Imbabura, and the use of their presence as an indicator of water quality. Secondly, I propose the analysis of their behavior when exposed to some natural products that could act as novel attractants or repellants. Thirdly, I evaluate the creation of a portable device for its control on the campus of Yachay Tech University, based on existing technology for the control of other species of flying insects. Finally, I recommend a simple line of research to determine if the attraction of simuliids to humans has a genetic or microbiological component that can serve to generate specific solutions for their biological control.

5 General and Specific Objectives

General objective

- Identify the species of black flies that live in the area of Urcuquí, Imbabura, Ecuador.

Specific objectives

- Develop control population methods based on their affinity or aversion to novel compounds.

- Evaluate integrated control alternatives.

- Understand the interactions between this species and animals (especially humans), including genetic and microbiological factors.

6 Materials and methods

6.1 Place of study

One of the endemic areas for simuliid-related diseases are the provinces of Esmeraldas and Pichincha (35), in close proximity to Urcuqui. The canton of San Miguel de Urcuqui is located in the province of Imbabura at 2384 mamsl and has an area of 767 km^2. Its temperature ranges between 14 and 22 °C, and the landscape includes very scattered areas of shrub vegetation and its abundant riverbanks (36). In addition, large areas of crops such as corn, potatoes, wheat and sugarcane, among others can be found here. For these reasons, it is a favorable place to study black flies.

To delimit the study area, a 4 km radius was established to our place of residence at Yachay Tech University (Fig. 3). Thus, it was possible to cover an area of 12.56 km^2, that is, the minimum distance that the black fly can travel to find either its food or mates. It is females that use blood as food source and, thus, must come back to flowing water to lay their eggs. In addition, since the campus Yachay extends along a depression towards the valley, I decided to sample black flies at three different altitudes to find out whether there were different species inhabiting niches at those altitudes.

Figure 3. *Aerial photo of the study site located in Urcuqui, Ibarra. Source: Google Maps*

6.2 Identification of the species of simuliids

For the identification of the simuliid species collected in the field, preparations were made based on known protocols and taxonomic keys. There are separate taxonomic keys for adults (female and male), pupae and larvae, using both macro and microscopic characteristics for the identification of these Diptera (Coscarón-Arias., 2003). Flies were captured and observed under a stereo microscope Leica EZ4 at the Biology labs of Yachay Tech University or with a Handheld Digital microscope (Celestron, LLC.), privately owned.

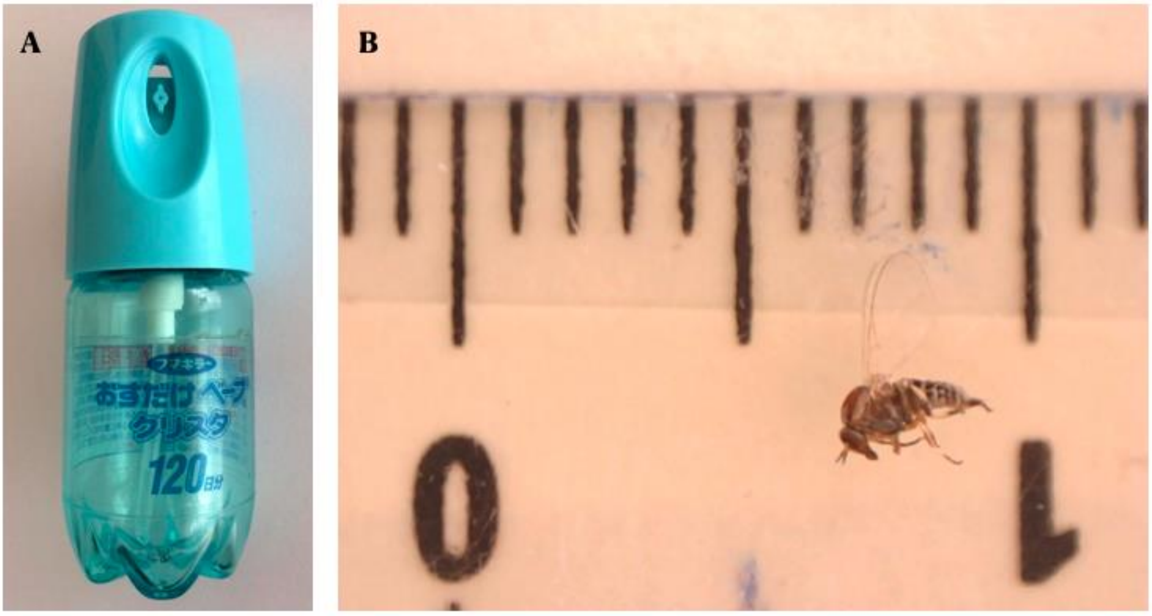

Figure 4. A, Japanese insecticide; B, Typical size of black fly in milimeters.

Twenty flies were obtained once they were attached to the skin during their bite of a volunteer. They were placed in a 15 ml tube and the interior was sprayed with a little Japanese insecticide whose main component is the pyrethroid insecticide transfluthrin (Fig. 4A). Once they stopped moving, they were observed under a microscope for identification (Fig. 4B). Although initially the author of the present study was confident about the proper identification of the species, the jargon utilized in the keys is complicated and it was decided to ask experts' help to properly determine the species. See in Fig. 5 on how similar some anatomical features in the captured flies are compared to those described in the keys of Coscarón-Arias, 2003.

Figure 5. Left, black fly captured on Yachay campus. Right, capture from a key in Coscarón-Arias, 2003, in which a similar thorax (escudo) is described, belonging to the species Simulium oyapockense.

Another complication for the determination of the species through keys is the fact that some of the steps require the dissection of the larvae of the simuliIds. This step was difficult to achieve due to the lack of mobility of the author to the study area due to the coronavirus pandemic. It is also possible to associate any flying black fly on campus with the wrong larvae, and vice versa.

Another way to determine the species of black flies is through the sequencing of conserved genes such as the ribosomal subunit 18S and the COI gene (Cytochrome oxydase subunit 1 (37). Taking advantage of a sequencing of black flies in the laboratory of a colleague of a Yachay professor, it was determined that one of the possible species on campus was *Simulium veracruzanum*.

Performing a nucleotide BLAST search in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (37) leads to a high probability of the organism being *S. veracruzanum.* Although the data is very interesting and based on very powerful molecular genetics technologies, there is no evidence of the presence of such species in Ecuador prior to this date. Hence, it is important to verify through the expertise of a specialist in black flies whether this is true.

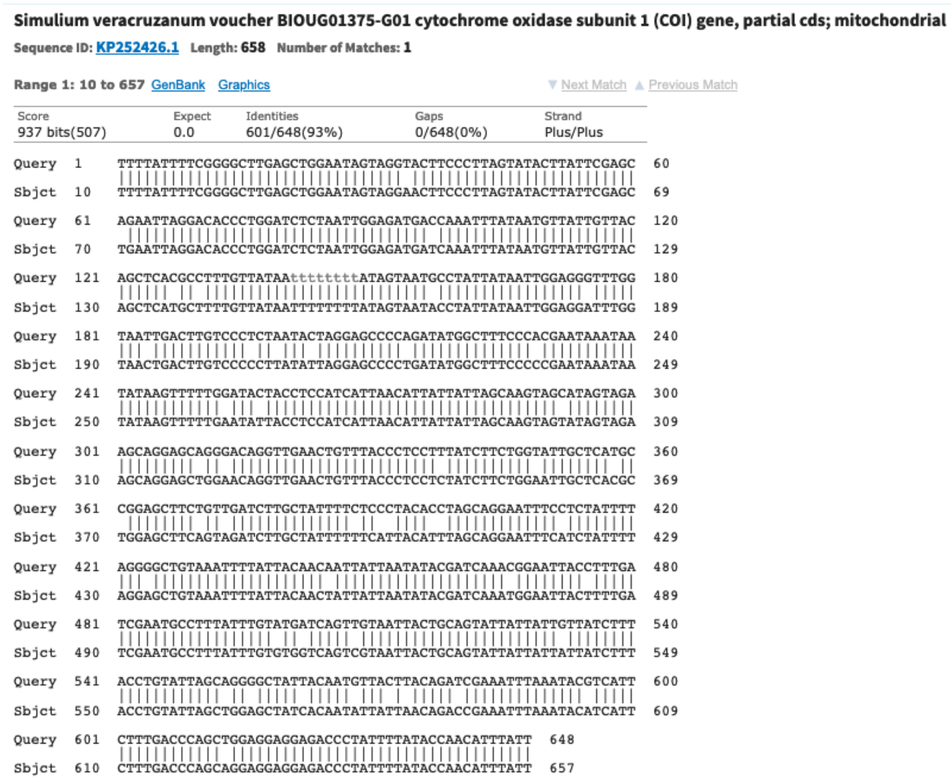

Figure 6. Outcome of Blast search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) performed after Sanger-sequencing the COI gene of a black fly captured on Yachay campus.

6.3 Attractants and repellents

With the purpose in mind of hopefully finding novel attractants and repellents that work with the black flies in the valley of Urcuqui, it was important first to acknowledge previous research. How have humans been able to repel flies through their own body chemistry? This area of research opens new possibilities that would include the recruitment of locals and non-locals as subjects for a study.

6.3.1 Human factors: personal odors and skin microbiota

The microbiota of the skin plays an influential role in making victims more attractive to insect bites by the production of certain odors, such as increased levels of ammonia in sweat (38, 39). Furthermore, microorganisms housed on human skin produce volatile organic compounds, such as aliphatic carboxylic acids (40). On the other hand, the different types of glands that animals themselves have are responsible for the distribution and secretion of certain substances. For example, the apocrine glands, which play an important role in chemical communication have different locations and functions; however, they all secrete substances composed of proteins, lipids and steroids (41, 42). In addition, the eccrine glands produce odorless sweat, composed of proteins, urea, amino acids, lactic acid, ammonia, some salts, and water. Therefore, the bacteria present here are responsible for initiating the odor conversion. The microbiota of vertebrates is poorly studied, unlike that of humans. In humans, bacteria of the genera *Corynebacterium*, *Propionibacterium*, and *Staphylococcus* break down the substances secreted by the glands to convert them into other substances, mainly carboxylic acids (41, 42, 43).

According to Verocai et al. (2017), *S. vittatum* females seek their host through substances such as 2-heptanone and naphthalene (44). 2-Heptanone is present in cattle (45), and naphthalene is found in humans, livestocks, and dogs. (46, 47, 48) These compounds elicit responses in hematophagous dipterans, as well as non-hematophages ones, which are considered pests that bite livestock excessively to suck their blood (45). The response of simuliids to other chemical solutions such as 1-octen-3-ol hexane, 1-octanol, and acetophenone has also been studies (49). The latter compound has been reported as an electrostimulator or as an attractant; additionally, it is associated with some species of mosquitoes (49).

6.3.2 Effect of Diet

Additional factors such as diet also influence the secretion of distinctive odors for each individual (50, 51). Where these distinctive odors make them preferred by biting insects. A study conducted in West Africa showed that people who consume alcohol become more attractive to some species of mosquitoes. The action of alcohol in the human body is to increase secretions rich in acid. Furthermore, alcohol produces vasodilation, which increases the internal diameter of the vessels, allowing more blood to flow, and consequently there is a slight increase in skin temperature (52). A similar example is provided by pregnant women. They are preferred by insects mainly because of their increased body temperature and greater rates of respiration (53). Therefore, it can be said that insects that feed on blood are attracted to odors emanating from the body and to the host's body temperature.

6.3.3 Effect of the Environment

Studies on insect responses to different colors begin with von Frisch's experiments on bees (54). While, the experiments of Bradbury and Bennett (1973) on color and shape preferences in certain species of simuliids, defined an order of preference from black to blue, red, white and yellow colors. This study notes that the reflectivity of the incident light is inversely proportional to the degree of luminosity. In other words, the lower the reflectance, the greater the attraction. According to Brancken et al. (1962) yellow and green colors are more attractive to insects, since they resemble the colors of nature. In another study, Brancken shows that flies prefer the colors black, blue and red, because of their perception of a small range in the visible spectrum (56). Finally, Peschken and Thorsteinson (1965) reported that these insects were attracted to colors with wavelengths in the range 380-550 nm.

On the other hand, the simuliids studied tended to land near the corners of squares and rectangles, and the points of stars and triangles. This means that these insects focus on points of convergence of contours on the targets (55). However, Davies (1961) observed that simuliids land more frequently on silhouettes with low reflectance (57). In addition, simuliids are more attracted to solids and solid objects such as triangles, circles, squares, etc., as opposed to silhouettes with broken outlines (58).

6.3.4. Natural products

In the area subject of study the locals mention the power of some types of plants for the purpose of either repelling or attracting black flies. However, there are no studies on that matter and those stories could well be a matter of superstition. Still, it would be interesting to scientifically test all the claims by local inhabitants about different plants to determine the veracity of the information. The next segment proposes a way to test those claims.

6.3.5. Design of a device for determining the response of black flies to attractants and repellents

For the purpose of testing whether human secretions, other substances or secondary metabolites of plants could serve as attractants or repellents for the black flies, a simple device was constructed (Fig. 7). A transparent plastic tube was designed with a hole at one end to place the attractants or repellants and another for the introduction of black flies. Sufficient repetitions should be performed for each secretion, substance or plant extract. The presence of flies closer to the element to be tested at the end of each repetition would mean that it has an attractive action. The presence of flies further from the element to be tested means that it has a repellent action. The data would be analyzed and processed with suitable statistical tests for its validation.

Figure 7. *Prototype for analysis of simuliids preferences.The control smell is a neutral smell known to not elicit any particular response by the black flies.*

6.3.6. Determination of genetic or microbiological components in the attraction of flies to humans

To determine the possible existence of genetic components associated with the attraction of simuliids by humans, the following process was proposed:

1. Preparation of an informed consent form and application to an ethics committee for the approval of the study, since it would involve taking saliva samples from humans. Importantly, no live human cells would be kept in the study.

2. Generation of an online survey for students and other personnel of the Yachay Tech campus or street surveys of residents of the town of San Miguel de Urcuquí.

3. Once the results have been processed, the individuals participating in the survey would be contacted and divided into two groups: those who are rarely bitten by flies and those who are constantly bitten by a large number of flies. Those who decided to participate in the study would be asked to fill out the informed consent form.

4. A saliva sample would be obtained from individuals in both groups, always maintaining the anonymity of the participants. A skin microbiota sample would also be obtained by rubbing cotton swabs on the skin of study participants and immersing said swab in a saline solution. One such sample could also be used in the odors preferences apparatus mentioned in the previous section (6.3.5).

5. DNA would be extracted from the samples, following established protocols (Lum et al., 1998), in the Biology laboratories of Yachay Tech University. A part of the skin microbiota samples would be frozen at -80 ºC in a final concentration 15% glycerol.

6. Genes already involved in the attraction of mosquitoes to humans would be sequenced. Also, the ribosomal genes of the skin microbiota sample would be sequenced to determine which species they contain. This part is the most costly part of the project (59).

Since the application and approval of this part of the study by an ethics committee accredited by the Ministry of Public Health of Ecuador may take about three months, it was decided to simply design this part of the project without executing it at the time of writing this thesis. In addition, the current pandemic and lack of funds for sequencing of the genetic material obtained precluded following through with the experimental part.

6.4 Development of an electronic device for the local elimination of black flies and their counting

For anyone who visits the Yachay campus, the annoyance of biting flies is evident: as soon as one stops walking, a cloud of flying simuliids surrounds one body and painful bites accrue. As part of this thesis it was decided to design a portable device that served both to gain information about the amounts of flies on campus and also suggest ways to eliminate them.

Hence, for the proposed objective, the development of the device was divided into three fundamental steps:

1. Simulation of a circuit for counting insects.

2. Implementation of the circuit in a fly-trapping device.

3. Design of the autonomous support system of the device.

6.4.1 Traps

There are different types of fly traps, but there are no specific ones for simuliids. Below a list with some of the most used traps to control the black flies is provided.

6.4.2 Electric Racket

The first device is the most used one in homes in Urcuquí, the electric racket (Fig.8). It is portable and has a design similar to a tennis racket. This device has a handle with a high voltage generator. Its circuit is made up of a battery, a transformer, an electronic oscillator, and a high voltage multiplier. Where the fly closes the circuit and a capacitor connected to the electrodes generates a spark, it causes the death of the fly (60).

Figure 8. *Electric Racket (55)*

6.4.3 BG- Sentinel

A trap produced by Biogents (Fig. 9), one of the most used ones by biologists on the planet, is the BG-Sentinel or BG trap or the BGS trap. This plot is a trap for mosquitoes for example: *Stegomyia* spp. and *Culex quinquefasciatus*. Also, this trap can be used with or without CO_2 as an attractant (61).

Figure 9. *BG- Sentinel (56)*

6.4.4 BG-Lure

Another of Biogents' devices is the BG-Lure (Fig. 10). Unlike the one mentioned above, this one uses an artificial scent from human skin. This substance is a mixture of ammonia, lactic acid and caproic acid in amounts that provide an excellent emission profile that is particularly attractive to mosquitoes (62).

Figure 10. *BG- Lure (57*

6.4.5 CDC White Light Tramp

In addition, another US-made mosquito collection trap is the CDC white light trap (Fig. 11). This device consists of a photoelectric cell, a 6 volt battery, a fan to attract most common mosquito species and store them in a chamber (63).

Figure 11. *DCD White light tramp (58)*

6.4.6 Black light tramp (UV)

Finally, the black light (UV) trap has a similar operation to the CDC traps (Fig. 12), Unlike the CDC traps it includes a black light tube holder and ballast. The black light (UV) attracts a greater number and diversity of species of mosquitoes, culicoides and sandflies. Thus, it has a greater field of control towards insects. (64).

Figure 12. *Black light tramp (59)*

6.5 Simulation of a circuit for counting insects.

As part of this thesis it was important to gather statistics about black flies populations. Hence, a modified trapping device was developed that would also determine the number of insects killed. This would help the insects have more interest in the trap than in the animals and humans nearby, eliminating them while gathering important information about the population of black flies (numbers, seasonality, variations depending on climatic conditions, etc).

6.5.1 Theoretical model of the fly trap device

With the information presented on the traps above, I can conclude that they use mostly non-specific attractants for black flies, but may include substances such as CO_2, ammonia, lactic acid and caproic acid. Meanwhile, these devices mostly use light colors for their outer cask. Based on this, an electronic device was designed to catch the fly and streamline the fly counting process.

The proposed device would be suitable for places with a great flux of people such as bus stops, parks, sports complexes, etc. For this reason, it would require its own power system. In addition, this system should be sustainable and friendly to the environment. As Ecuador is a country with much sunshine, solar panels will be a good option.

The first prototype of this device consists of an infrared sensor (IR) is required to detect an incoming fly to device. There are several types of sensors such as active, passive, modulated, reflective, slot, scanning, among others (65). For our interests the use of a reflective optical sensor is indicated. Also, the choice of the type of infrared to use was based for two criteria. First, the prices of the sensors that exist in the market were compared and secondly, the stopping distances of the objects were evaluated. The final choice was the CNY70 phototransistor. Another option would be a sound sensor that counted flies any time an electric discharge took place.

6.5.2 CNY70 simulation in Proteus

The CNY70 is a reflective optical sensor with a phototransistor output, infrared light, with a filter for visible light, with a range between 0 mm to 5 mm. This small cube-shaped device has four legs, the first two are those of the anode and the cathode, the others correspond to the collector and the emitter of the receiver, the latter are 2.8 mm apart (Fig.13) (66).

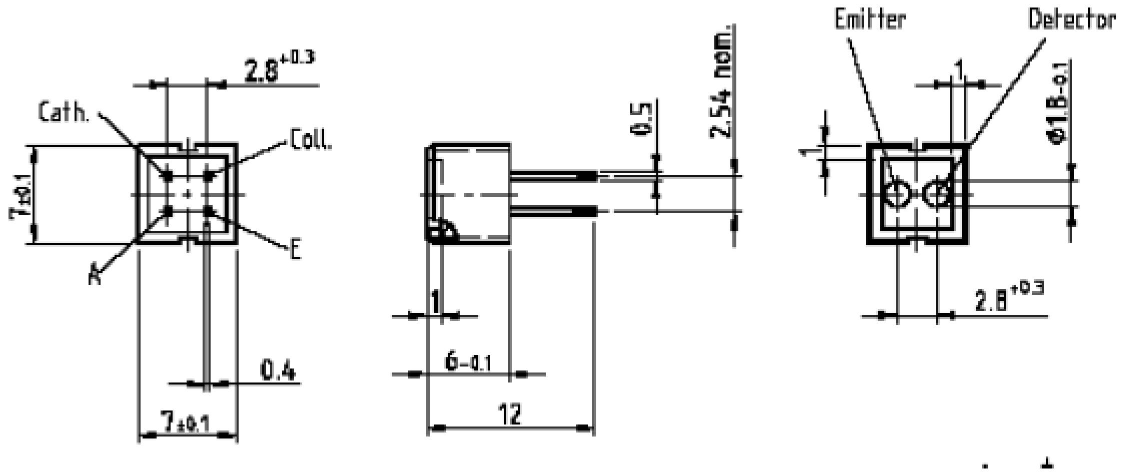

Figure 13. *CNY70 phototransistor dimensions in millimeters (66).*

The operation of this device is by means of the emission of an infrared signal by the transmitter, which, if an object is crossed, carries the reflection to the receiver and thus activates the circuit recorded with the Proteus 8 professional software. Figure 14 shows the circuit attached to a 12 V supply. Resistance must be greater than 220 Ohm, although a lower voltage battery can also be used and the resistances can be varied. The emitter LED light has the same qualities as a conventional led; that is, while it is powered, it fulfills the function of emitting infrared light. The LED of the receiver is connected to other accessories (potentiometer, resistors, and amplifier) for proper operation.

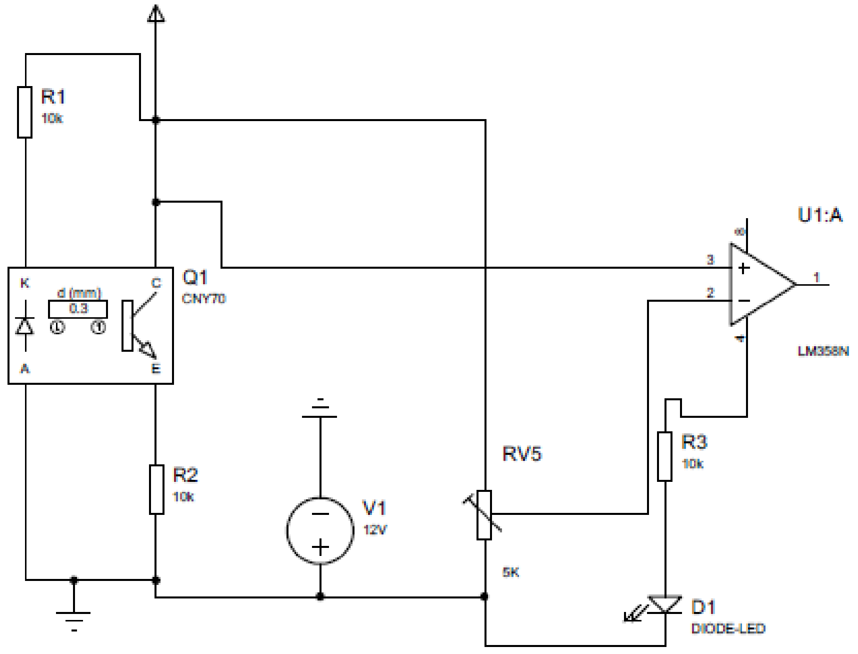

Figure 14. CNY70 Test Circuit Schematic.

6.5.3 74LS90 Integrated circuit

A 74LS90 is an integrated circuit counter that generates a count from 0 to 9 in a cyclical and natural way. This consists of four bits which are QA, QB. QC and QD. This is a decade counter with a binary BCD output, which adds one unit for each pulse it receives, displaying a binary number from 0 (0000) to 9 (1001). Additionally, it has the facility to be coupled with others of the same type to count more digits (67).

6.5.4 74LS47

The 74LS47 is a decoder which is responsible for receiving the binary number delivered by the counter and transforming the BCD code. This generates a signal which activates the LED segments responsible for each number. Working in addition to the display with a LOW output (logical 0) it will illuminate a segment of LED while a HIGH output (logical 1) will turn it off (67).

6.5.5 7 Segment display

The seven segment display is one of the ways of representing characters in electronic equipment. Meanwhile, as its name indicates, it has seven segments that can be turned on or off individually. This device is made up of LEDs in such a way that it forms the number 8. These LEDs, when supplied with energy, give us an output as a number or letter. There are two types such as common anode and common cathode, that is, all anodes or cathodes joined to a single terminal, respectively (67).

6.5.6 Final counting circuit

All these previously mentioned elements are to be connected with the corresponding pins for data capture in the Proteus software (Fig. 15 and Fig. 16).

Counter

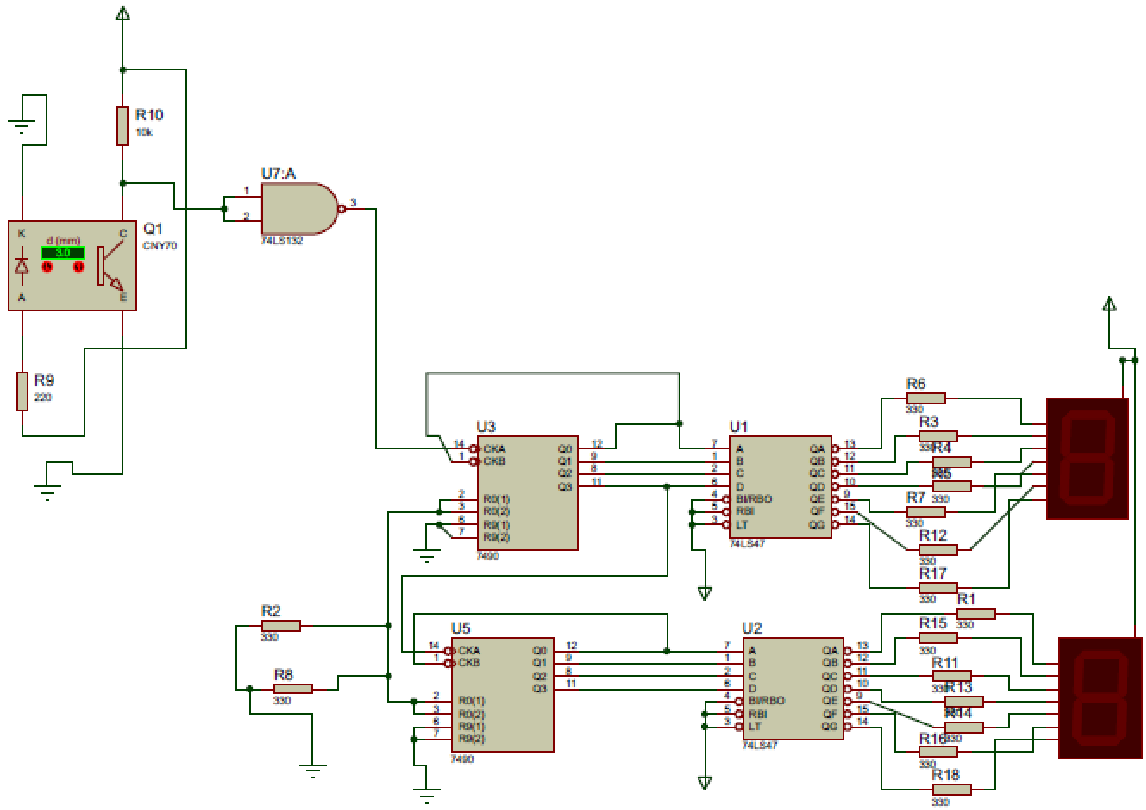

Figure 15. Schematic diagram of the circuit

Electric mesh

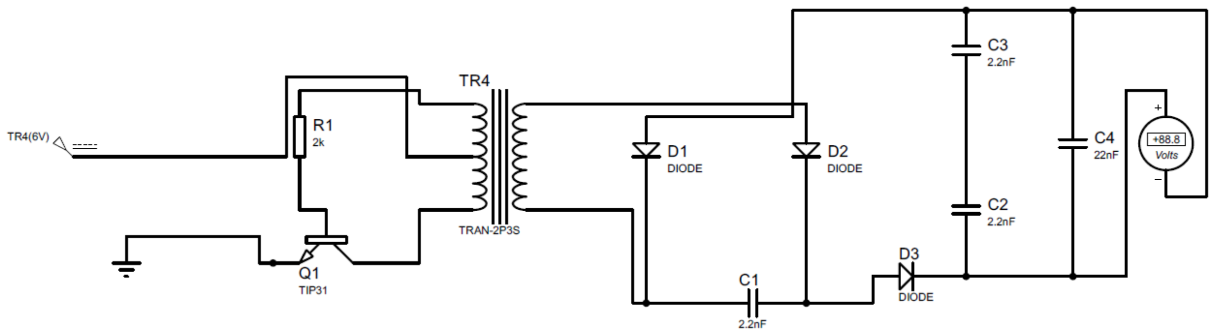

Figure 16. *Schematic transformer circuit of 6V to 88.8 V*

6.5.7 Discussion on fly trap device

The proposed counting circuit is based infrared optical sensing sensor proposed above, in such a way that an ascending count can be made from 0 to 99. As we are going to connect an analog sensor we must take into account the possibility of rebound and eliminate it. It is worth mentioning that this device only works as a counter so it is not capable of showing results from infrared, temperature, humidity or related signals.

This device is triggered if an object moves directly in front the photodiode and the phototransistor. A bounce pulse is generated and this internally saturates the phototransistor, thus generating an output pulse that will go to the 74LS132 where a bounce is subsequently eliminated. Later this signal passes to the CLK of the 74LS90. This is an asynchronous decade counter with BCD output in binary. Additionally, we add two 330 Ohm resistors in order for the counter to start from 00. Hence, it works in conjunction with the decoder or BCD-Conductor known as 74LS47, which consists of seven segments with low active outputs. These outputs are conducted towards the seven segment display showing the number of objects that passed through the sensor.

Figure 17 shows a proposal for the structure of the fly trapping device with its respective dimensions. In addition, all device are integrated inside prototype; and preferred substances of the simuliids can be placed inside it. Also, we can see that it complies with the above characteristics to make it attractive to these insects such as color and shape.

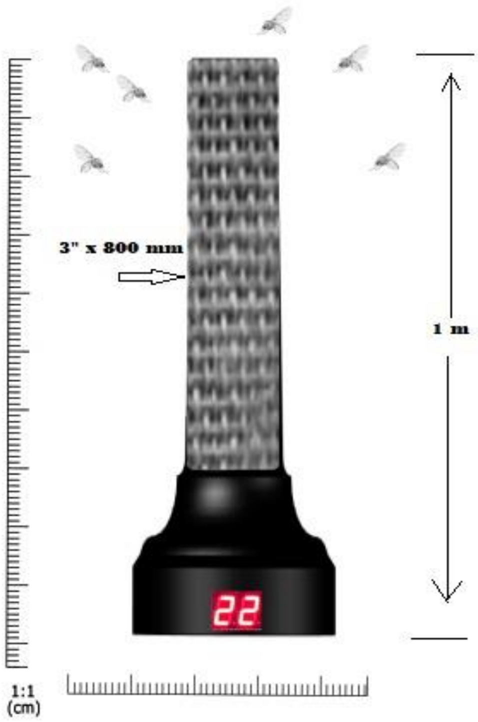

Figure 17. *Final prototype structure*

7 Conclusions and future directions

In the present work it has been proposed several ways to tackle an important problem for the health and well-being of human and animal residents of the valley of Urcuqui. Black flies are present in an area, because there are clean enough water channels. They reach nearby populated sectors thanks to their flying capacity. Knowing the need of the female flies for clean water to lay their eggs in represents an opportunity for the biocontrol of this pest. The one likely species found through gene sequencing, *S. veracruzanum,* has not been previously described in this country, although its role in disease transmission is known from studies in neighboring countries. Therefore, it is important to determine whether this species is indeed *S. veracruzanum*, and whether it is the only simuliid living in the area.

Since black flies are insects adapted to humans and animals, I proposed ways to study the attractants and repellents present in humans or in plants, reported by locals as protective against black flies. A simple device made of plastic was designed to test different substances. A study aimed at finding differences in the attractiveness of inhabitants of the valley compared to outsiders due to genetics or skin microbiota was proposed.

Finally, based on designs of existing fly traps I proposed the design for a black fly trap using various electronic components that is easy to use. These traps could be placed in various locations on the university campus to both eliminate flies and help assessing local fly populations. Furthermore, preventive actions against black fly bites should also be taken into account. Thus, behavioral adjustments and urban planning could help mitigate the local fly problem. For instance, it should be avoided to build houses too close to rivers.

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