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GUIDELINES FOR SAMPLING AND PRESERVATION OF BLACK FLIES (DIPTERA: SIMULIIDAE) – AN ATTEMPT TO STANDARDIZE FIELD AND LABORATORY PROCEDURES

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Abstract

This paper provides guidelines for sampling and preserving black flies (Diptera, Simuliidae), aiming to standardize field and laboratory procedures. The authors detail the methods for processing black fly samples, from collection to identification, for each stage. They also propose a sampling protocol that considers various environmental factors influencing species distribution and abundance. The paper is based on the authors' personal experience and aims to contribute to a broader understanding of black fly ecology and taxonomy.

KEY WORDS: black flies, Diptera, Simuliidae, sampling protocol, ecology

Introduction

The study of black flies (Diptera, Simuliidae) requires an accurate methodological approach in the field and the laboratory due to the fragility of the imago, its small size, and the difficulties associated with searching for breeding sites. A distinctive characteristic of black flies is the blood-feeding behavior exhibited by adult females. Preimaginal stages inhabit running waters with specific environmental conditions for the species. Various factors significantly influence the composition of Simuliidae fauna in a biotope. These include the type of running-water habitat and its structural features, such as the chemical components of the water, the level of shading, the types of aquatic and riparian vegetation present, and the presence of natural black fly enemies.

Methods of black fly sampling have been published in different publications: as part of books (Rubtsov, 1956b; Usova, 1961; Kaplich & Skulovets, 2000; Adler *et al*., 2004; Chubareva & Petrova, 2008), as

methodological guidelines (Rubtsov, 1956a; Service, 1977), scientific articles related to sampling (Khalin *et al*., 2021) and statistical data processing (McCreadie *et al*., 2006).

This paper proposes a sampling protocol and describes methods for processing black fly samples for each stage (adults, larvae, and pupae), from collection to identification, based on our personal experience. Only the methods necessary for black fly identification based on morphology and expanding knowledge about species ecology are given in the article.

Sampling

Black flies demonstrate uniformity in their morphological traits, creating taxonomical issues in the identification. The most important stage for species identification is the male imago since the shape of their external genitalia is evolutionarily distinct and specific for each species. Conversely, the morphological features of female genitalia are more homogenous and can pose problems in identification to the species level. A combination of morphological traits is applied in the identification of females. These include the genitalia structure, claw shape, coloration, pubescence and shape of frons, head appendages, coloration, length and width ratio between different segments of the legs, coloration, pattern and pubescence of scutum, and wing venation.

The lifespan of males is short; they live only a few days (Crosskey, 1990). It is common to rear Simuliidae imagos from pupae to get males. Alternatively, a sweeping net can be used. Sweeping vegetation along the banks of streams during black fly adult emergence and swarming provides decent results. Creeks and rivers are often bushy, and the sweeping net serves longer if it has an additional protective sweep net bag with dense fabric.

Spider webs along streams are a good source for collecting adult Simuliidae (Jensen, 2015). In one case, more than 70 imagos, mostly males, were collected from a single spider web. Visiting black fly breeding sites (i.e. running water) should be planned before a field trip. Problems that may arise and lead to failures in the search for black fly are the following: the drying-up and slowing-down of water flow; changes in the landscape; dirty water with a high turbidity; water polluted by regular waste discharge from industrial companies, etc. Water can also be contaminated by agricultural activity or human garbage disposal. Satellite maps are used to find suitable locations for sampling. The most suitable sites for black fly sampling are stream meanders. The bending of a watercourse is a good guarantee of avoiding stagnant water sites along the stream. The researcher should cover different types of watercourses in the planned district or region and include crenal, rhithral, and potamal zones in the selection. Sites for black fly preimaginal stage sampling should be accessible to the researcher. If sampling is planned in a Natural Reserve, permission must be obtained beforehand. Information concerning the locations of creeks and the conditions of rivers can be sourced from the local residents or news reports.

Pupae are collected in containers for further rearing. It is better to select darkened pupae that represent a pharate form of adults ready to emerge. Pupae are located in a silky cocoon, attached to a substrate immersed in the water, such as bark, leaves, sticks, different parts of riparian vegetation, human-generated waste (bottles, different types of plastic, glass, tires, etc.), or stones of various sizes. In the case of a rocky bottom, pupae can be found directly attached to the bottom. Collecting them with the substrate is best to avoid injuring them. When this is not possible (a large stone, glass bottle, large piece of plastic or another difficult-to-carry substrate), the pupa should be detached from the substrate with thin-tipped hard tweezers (Fig. 1A). The pupa should be taken from the posterior part of the cocoon with care, without touching the posterior part of the pupa and injuring it. It is difficult to predict the pupation and emergence time in a prospective breeding locality. Not all species at a stream site pupate at the same time. Additionally, pupal

specimens may sustain injuries during other stages of processing. Consequently, larvae are a good source to establish faunal composition since they can be found almost all year round.

Larvae collected together with pupae are sorted and placed in the appropriate preservation liquid, depending on the purpose of the investigation. They can be placed in 70% ethanol for morphological identification and 96% ethanol for molecular identification or in Carnoy's solution (1 part glacial acetic acid, 3 parts 96% ethanol) for polytene chromosome conservation for cytological identification. To obtain clean samples, the preservation liquid should be replaced a few times soon after collecting samples in the field and later in the laboratory. Samples for molecular and cytological identification should be stored in a deep freezer, maintaining a temperature range between -20°C and -80°C. The exact number of larval stages is uncertain, and only last-instar larvae are used for precise identification. Last-stage larvae can be recognized by the darkened paired pupal gill buds located on the lateral sides of the thorax, behind the head (Fig. 2D). It is important to maintain the larvae and pupae in humid conditions when they are not immersed in water. It is difficult to keep larvae alive and undamaged in hot weather, in which they die and decompose quickly. Consequently, cooling must be used to avoid larval decomposition if it is impossible to place them in alcohol immediately in the field.

Collecting water from the stream where the sampling is done is essential. This water will be used to create humid conditions during the rearing of the collected pupae. The best places for sampling larvae and pupae are sunny stream sections with the highest flow velocity and abundant substrates to which larvae and pupae can attach themselves. Larvae are mainly found in such places, while pupae prefer stiller water, closer to the banks.

To process statistical data, it is necessary to determine the number of specimens collected within a specified area. An area of one square decimeter (dm^2) is commonly used to measure the abundance of preimaginal stages in a waterbody (Yankovsky, 1979; Kaplich & Skulovets, 2000; Petrozhitskaya & Rodkina, 2007), although a square foot has been used (Sommerman *et al*., 1955). We consider 1 dm^2 more practical. In order to ensure accurate measurements, a frame cut from a plastic dish is used to represent a square decimeter (Fig. 1E).

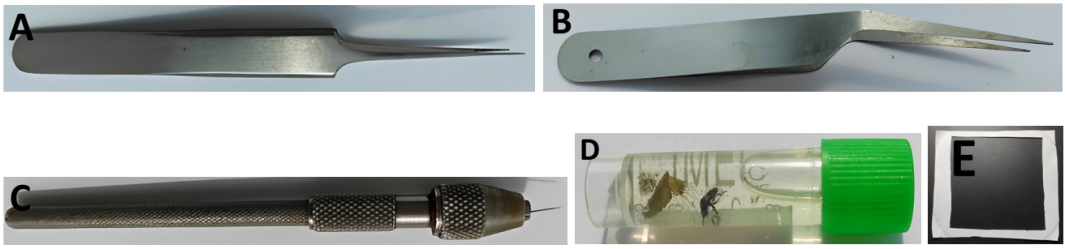

Figure 1. Sampling of black flies. Tools and results: A – hard tweezers; B – soft tweezers; C – dissecting needle; D – results of individual rearing; E – frame for 1 dm^2 measurement.

Sampling protocol

Detailed collecting data should accompany sampled material to obtain comprehensive environmental information. A sampling protocol collects, organizes, and manages data from a sampling point in field conditions. Taking into account abiotic and biotic factors allows us to predict the ecological factors that affect species distribution, population abundance, and the formation of Simuliidae assemblages. We propose a sampling protocol for collecting black flies in the field.

The protocol (Table I) is divided into sections: heading, sampling area, waterbody and environment, observed stages, land, and remarks. The "Heading" section requires data about the date, time, and, if necessary, the note taker. The "Sampling area" section provides specific fields related to coordinates, altitude, country, district, city or village, code, and a field for additional information called "Other". The "Waterbody and environment" section contains fields for the waterbody type, chemical, and physical parameters, the features of the waterbody and its banks, as well as the animal and plant co-inhabitants. The "Observed stages" section allows the recording of the stages found, the substrate, and methods of specimen sampling. The "Land" section is for a description of the land use near the sampling point and the surrounding landscape.

The rating system used for the "Observed stages" and "Bottom of waterbody" sections is based on the abundance of specific elements, where: 1 = sparse presence (element is barely present, represents less than 10% of the total composition), 2 = minimal presence (element is present but not dominant, represents about 10% to 25% of the total composition), 3 = moderate presence (element is fairly common, represents about 25% to 50% of the total composition, 4 = high presence (element is abundant, represents about 50% to 75% of the total composition), and 5 = dominant presence (element is the primary component, represents over 75% of the total composition).

Labeling

All samples must be labeled as soon as they are collected. The label should include the date and location of sampling; the collector's name is optional. Once the sampling protocol is completed, each sample is given a unique code, which is then written on the label. For ease and efficiency, we recommend using a code format composed of three numbers interspersed with dots:

The first number represents the year of sampling. This helps the sorting of samples collected across different years.

The second number is the specific sample number taken at a particular time and place during the given year. This ensures that each sample taken within a year is unique.

The third number denotes the sample sequence in a specific location and time frame.

An example of a code might look like this: "2023.5.3", where "2023" represents the year of sampling, "5" is the specific sample number taken that year, and "3" stands for the third sample taken at that particular location and time.

Pupae rearing

Individual rearing is the most effective way for black fly identification because pupa and imago features can be applied together for identification. Each pupa should be individually placed in its own plastic tube, along with the substrate to which it is attached. If pupae are very close to each other and cannot be separated, up to three pupae can be placed in the same tube. If several pupae are placed in a tube, they should be checked to see whether they have a pharate form of imago inside. Pupae that contain only exuviae should be removed. Several drops of water should be added to the tube to wet the pupa and create humid conditions for pupal development. The best results were obtained when we used water from the location where the pupa was collected. Typically, the tube is closed with a cap, which is generally sufficient to maintain a pupa until the imago emergence. Daily care of a tube includes opening it for fresh air, removing excess water, adding a drop of water if it is drying out, introducing ethanol if the pupa appears to be dead, or moving it to a different tube if necessary. Alternatively, the tube can be closed with a dense cotton ball, which should be replaced daily with a new, moist one. However, this method might present difficulties during expeditions or when handling large sample sizes.

Table I. Simuliidae sampling protocol.

Note taker:		Date:	Time:	
Sampling area				
Coordinates:			Altitude:	
Country	District	City / Village	Code	Other
Water body and environment				
Type: River (name if): | Creek | Spring | Irrigation channel				
Width of waterbody (cm)	Length of analyzed part of waterbody (cm)	Depth of waterbody (cm)	Water flow velocity (m/s)	
Turbidity of waterbody	pH	TDS (total dissolved solids)	Dissolved oxygen (percent)	Temperature of waterbody (°C) / Temperature of air (°C)
Bottom of waterbody	Mud	Sand	Gravel	Stones / Sticks and leaves in water
Evaluation of abundance (from 1 to 5 or dash)				
Bank: Trees | Bushes | Grass (height: low | medium | high) | Riparian Vegetation | Naked Shade: Shaded | Semi-shaded | No shadow				
Identified plants		Identified invertebrates		
riparian vegetation	underwater	in air	in water	
Observed stages				
Stage	Eggs	Larvae	Larvae (last instar)	Pupae / Imagos
Evaluation of abundance (from 1 to 5 or dash)				
Type of substrate where immature stages were found: Branches in water | Leaves in water | Stones | Riparian Vegetation | Garbage Method of adult collection: Manually | Landing | Sweeping net | Aspirator | Trap (type)				
Land: Land use: Urban | Natural | Agricultural | Pasture Landscape: Desert | Forest | Hill | Meadow | Mountain | Plain | Steppe | Valley | Other				
Remarks:				

The tubes must be shielded from direct light and maintained at a consistent room temperature during transportation and in the laboratory. Each tube is checked daily for adult emergence. They are set aside when the imago emerges from the pupa or opened for a while to allow fresh air in. If the pupa appears to be dry, a drop of water should be added. Some of the pupae collected are dead or infected for various reasons, including fungi and bacterial or viral infections. They can be visually distinguished by plaque accumulation or the presence of a bad smell. Such pupae should be preserved in ethanol before they decompose.

When the imago emerges, the cocoon and pupal exuviae are removed from the tube. An empty pupa (exuviae with cocoon) is separated from its substrate and then placed in the same tube as the emerged adult (Fig. 1D). To achieve the natural coloration and hardening of an adult insect's integument, it is important to keep the adult alive for at least 8 to 12 hours after it emerges. Pupae and adults are fragile and transferred using special soft entomological tweezers (Fig. 1B).

Typically, the first imago emerges from the pupa within a day, but the highest frequency of emergence is observed within two to three days. The success rate for adult rearing ranges from 50% to 80%. The result depends on the temperature conditions during the season in which the sample was collected, as well as the stream where the sampling was conducted. Polluted streams are believed to contain a higher quantity of pathogens, which adversely affect the preimaginal life stages and result in poor outcomes for individual rearing. All unsuccessfully reared pupae containing a dark mature imago inside are also preserved in alcohol for future identification. The successful identification of a species can sometimes be accomplished by observing the morphology of the exuviae. For instance, *Simulium balcanicum* (Enderlein 1924), a common species found in the Mediterranean, can be readily identified by the number and arrangement of respiratory filaments on its pupal exuviae.

Preservation

Storing larvae and pharate adults in 70% ethanol is sufficient to inhibit decay. Specimens that are intended for subsequent molecular identification should be preserved in 96% ethanol. The larvae should not be densely packed into the tube, as this can dilute the concentration of alcohol. Formaldehyde is used to fix and preserve specimens (Palmer & O'Keeffe, 1995; Illéšová *et al*., 2008). However, it is not recommended due to its toxicity and potential to degrade DNA.

Mounting on microscope slides

Preparations are made for the purpose of species identification. If the preparation is not intended for long-term storage, it is placed in glycerol for further manipulations. Canada Balsam or Euparal® are commonly used as permanent mounting media. Euparal is preferred due to its convenience, as it avoids the necessity of subjecting specimens to various chemical treatments for dehydration and purification.

The sample material should initially be categorized by type: imago, larva, pupa, and results of individual rearing (imago and exuviae). Subsequently, the imago specimens are sorted based on their size and coloration, while larvae are sorted according to their head features and size. Sorting also includes consideration of pupal features such as the shape of the cocoon, the presence and shape of dorsal projections on the cocoon, the number of respiratory filaments, their arrangement on stems, and the stem lengths. Additional morphological characteristics typically used for species identification are also taken into account.

The following actions outline the procedure for dissecting and mounting the result of individual rearing for male specimens. It is important to note the features of the specimens that are lost after dissection. These features can be inputted in a software-based table like MS Excel. The table includes the following features for males: body length, antenna, and palp colorations. For pupae, the noted features include the length of the exuviae and ratio between the lengths of the respiratory filament and the body of the exuviae.

The imago's hind leg, head, and terminalia are dissected using the dissecting needle (Fig. 1C). These parts are placed in a tube containing 10% solution of KOH or NAOH for 24 h. After being lightened, they should be placed in distilled water for an hour and then transferred to 96% ethanol for at least one hour. Subsequently, they are transferred onto filter paper to remove the remaining liquid. Finally, the parts are placed on a pre-prepared slide with a thin layer of mounting media.

The male genitalia consist of several components (Fig. 2A). The main part, a ventral plate, maintains all construction and has two branches with hooks that attach to the gonopodites. The connection between two coxites is established from their posterior parts. The initial step in male genitalia dissection involves severing this connection. In certain taxonomic groups within the Simuliidae species, it is necessary to examine the ventral plate from both ventral and lateral perspectives, and occasionally, a caudal view may be required. It

can be beneficial to capture photographs of these views prior to mounting to aid in further identification. The ventral plate is completely separated from other genital parts to make its characteristics more distinguishable. The median sclerite, parameres, and X sternite are situated posteriorly to the ventral plate and should be separated to facilitate examination and identification. The dissected genitalia, hind leg, and head are arranged in the center and then covered with a coverslip. The small size and surface tension cause them to begin moving and rotating. They should be watched, as they can move beyond the boundaries of the coverslip. Light pressure is applied using firm tweezers to prevent misalignment or improper positioning of the genital parts once the movement ceases. The lightened hind leg is used to clarify and examine two features: the calcipala, which is a protrusion on the basitarsus, and the pedisulcus, a depression on the second tarsomere (Fig. 2E).

The hind, middle, and fore legs, as well as the wings of the imago, are detached, along with the respiratory filaments and abdomen parts from the exuviae associated with the imago. All these components are mounted under a second coverslip on the same slide. The abdominal section of the exuviae possesses various small hooks on both its dorsal and ventral sides. It is sliced laterally, detaching the dorsal and ventral sections, to reveal the arrangement of these hooks. Due to its thickness, the cocoon is placed under the second coverslip based on its unique characteristics and condition. The remaining part of the specimen is preserved in a tube with 96% ethanol for subsequent molecular biology processing.

Before dissection, the following characteristics of female specimens should be documented in the software-based table: body length, antenna and palps coloration, clypeus coloration, and scutum pubescence. The process of lightening the hind leg, head, and genitalia is the same for both sexes. The mouthparts of the female should be prepared. This is done by using one dissecting needle to hold the head while using another needle to extract the cibarium and labellum. The identification process occasionally depends on the shape of the cibarium and the presence of teeth on its surface. Dissecting the cibarium and labellum reveals the mandibles and maxillae, which are used for identification based on the shape of their cutting parts and the number of teeth on various sides. The preparation of female genitalia (Fig. 2B) is as follows: using a dissecting needle to hold the anterior part of the genitalia, the cerci can be gently pulled out until the branches of the genital fork become visible. Once all parts, including the ovipositor valves, genital fork with branches, paraprocts, cerci, and spermatheca, are exposed, they should be covered with the coverslip. The parts mounted under the second coverslip for the female imago are identical to those described above for the male second coverslip.

Before dissecting a larva, it is necessary to document several features. These include the body length, the typical coloration of the body, the type of rectal papillae (simple or complex), and the head coloration. The dorsal side of the head can have two distinct patterns: black spots on a bright background (a positive pattern) or white spots on a dark background (a negative pattern). The shape of the median spot should be recorded, which could be a line, a droplet, or a triangle.

The following morphological traits of the larval head are used for identification (Fig. 2C):

The number of rays in the cephalic fan, which is controlled by a muscle located on the stem responsible for managing the folding and unfolding of the fan. Applying pressure to this muscle enables the fan to unfold.

The ratio between the segments of the antenna.

The shape and distance between the teeth of the hypostomium.

The number, length, and shape of the teeth on the mandibles, including differences in serrations.

The arrangement and number of hypostomium setae.

The ratio between the height of the hypostomium, the postgenal bridge and the postgenal cleft.

The number of rows of hooks in the posterior circlet, along with the number of hooks per row (Fig. 2F).

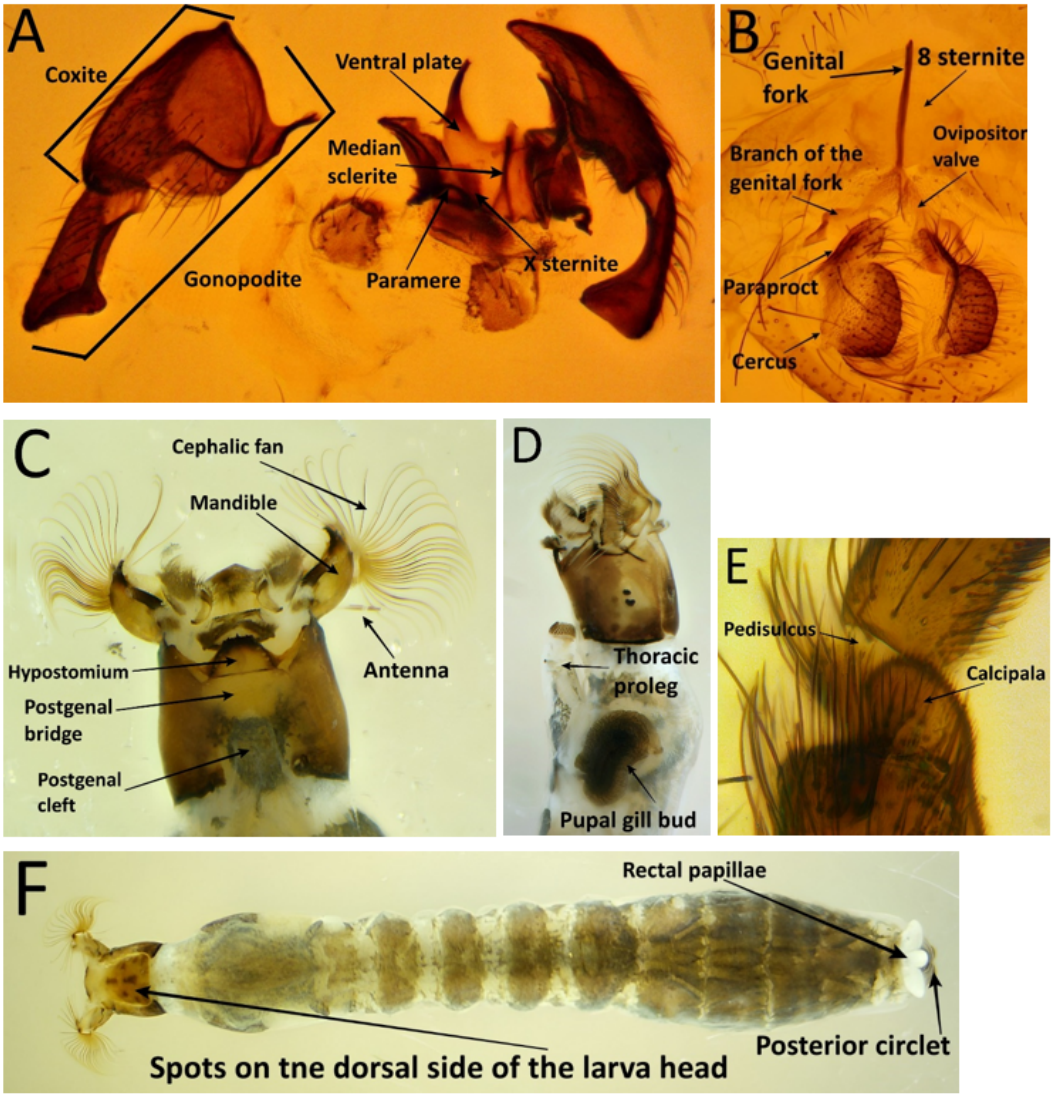

Figure 2. Morphological structure of Simuliidae life stages A – male genitalia; B – female genitalia; C – ventral side of the larva head; D – place of pupal gill bud; E – calcipala and pedisulcus; F – larva (full view).

The mounting process for a larva involves the following steps:

The pupal gill buds (Fig. 2D) are extracted from the thorax. To examine the number, thickness, and arrangement of respiratory filaments, they are untwisted using a dissecting needle. Placing the pupal gill

buds in 50% lactic acid for up to 30 seconds helps in uncoiling the respiratory filaments. Afterward, the pupal gill buds must be rinsed with distilled water.

The rectal papillae lobes (Fig. 2F) are white and can be either simple, consisting of 3 lobes, or complex with additional branching of these lobes. Due to retraction caused by ethanol, they can be inconspicuous. It is recommended to fix the slightly dried larvae in 96% alcohol to improve the visibility of rectal papillae.

The pupal gill buds and complex rectal papillae are mounted under the first coverslip on the slide.

The larva is cut into three parts. The head and posterior circlet are placed in 10% alkali for 24 h to lighten them, while the middle part of the larval body is stored in a tube with 96% ethanol for subsequent molecular identification. On the following day, the head and posterior circlet are transferred to a tube containing distilled water for 1 h, then placed in 96% ethanol for at least 1 h.

The head and posterior circlet are placed on mounting media on the same slide as the pupal gill buds and rectal papillae. Point ruptures are made on the lateral sides of the head using dissecting needles to detach the dorsal and ventral sides. The mouthparts behind it are removed for a clearer view of the hypostomium. Additionally, the mandibles are detached, and the cephalic fans are unfolded in order to count the rays. The posterior circlet is cleared to facilitate counting the rows of hooks. All the dissected parts are arranged in the center and covered with a coverslip. However, the small dissected parts can rotate and move out of the coverslip edges. They should be watched and adjusted to maintain the correct direction and inclination.

Mature pupae that fail to develop into adults can still provide useful information in determining the taxonomic composition of a sampling site, identifying rare species, and gathering general statistical data. However, it is impossible to accurately determine certain characteristics of immature adults within these pupae, such as coloration or wing venation. Additionally, the morphometric data derived from these specimens might lack precision.

The respiratory filaments and a segment of the pupal integument are detached from the pupa, and their lengths, as well as the length of the pupa itself, are recorded prior to dissection to calculate their ratio. The pupa, still containing the immature imago, is placed directly into an alkaline solution. The presence of exuviae has the potential to hinder the alkali-induced lightening of imago details. Therefore, it is recommended to perforate the pupa multiple times with a dissecting needle before placing it in the alkali solution. Following this, the remaining dissection steps for an immature adult are the same as for a pharate adult.

Conclusion

The methods presented in this article have undergone testing and practical application by the authors. They represent detailed, consistent guidelines for different stages, including sampling, data collection, rearing, preservation, labeling, and mounting. The proposed sampling protocol for black flies aims to facilitate and standardize fieldwork. Our recommendations have practical significance for Simuliidae researchers.

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References

Adler, P. H., Currie, D. C., & Wood, M. D. (2004). *The black flies (Simuliidae) of North America*. Comstock Pub. Associates, New York City, New York, USA, 940 pp. ISBN 0-8014-2498-4.

Chubareva, L. A., & Petrova, N. A. (2008). *Tsitologicheskiye karty politennykh khromosom i nekotoryye morfologicheskiye osobennosti krovososushchikh moshek Rossii i sopredel'nykh stran (Diptera,Simuliidae): atlas* (Cytological maps of polytene chromosomes and some morphological features of black flies of Russia and neighboring countries (Diptera, Simuliidae): atlas].Tovarishchestvo Nauchnykh Izdaniy KMK, Saint-Petersburg–Moscow, Russia, 354 pp. ISBN 978-5-87317-471-3. [In Russian].

Crosskey, R. W. (1990). *The natural history of blackflies*. John Wiley & Sons Ltd, Chichester, UK, 711 pp. ISBN 978-0471927556.

Illéšová, D., Halgoš, J., & Krno, I. J. (2008). Blackfly assemblages (Diptera, Simuliidae) of the Carpathian river: habitat characteristics, longitudinal zonation and eutrophication. *Hydrobiologia*, *598*(1), 163-174. DOI:10.1007/s10750-007-9148-4.

Jensen, F. (2015). *Simuliidae (Black-flies). The Greenland Entomofauna: An Identification Manual of Insects, Spiders and their Allies*. Brill, Boston. USA. 417-425. ISBN: 978-90-04-25640-8.

Kaplich, V. M., & Skulovets, M. V. (2000). *Krovososushchiye moshki (Diptera, Simuliidae) Belarusi* (Blood-feeding black flies (Diptera, Simuliidae) of Belarus). BGPU by M. Tanka, Minsk, Belarus, 365 pp. ISBN 985-435-264-1. [In Russian].

Khalin A. V., Aibulatov S. V., & Przhiboro A. A. (2021). Sampling Techniques for Bloodsucking Dipterans (Diptera: Culicidae, Simuliidae, Ceratopogonidae, Tabanidae). *Entomological Review*, *101*(9), 1219-1243. DOI: 10.1134/S0013873821090013.

McCreadie J. W., Adler P. H., Grillet M. E., & Hamada N. (2006). Sampling and statistics in understanding distributions of black fly larvae (Diptera: Simuliidae). *Acta Entomologica Serbica*, *11*, 89-96.

Palmer R., & O'Keeffe J. A. Y. (1995). Distribution and abundance of blackflies (Diptera: Simuliidae) in relation to impoundments in the Buffalo River, Eastern Cape, South Africa. *Freshwater Biology*, *33*(1), 109-118. DOI: 10.1111/j.1365-2427.1995.tb00391.x.

Petrozhitskaya L. V., & Rodkina V. I. (2007). Taxonomic composition and landscape-biotopical distribution of black flies (Diptera: Simuliidae) in the Khemchik river basin (western Tuva). *Parazitologiya*, *41*(4), 241-252.

Rubtsov I. A. (1956a). Metody izucheniya moshek (Methods of studying black flies). AN SSSR, Moscow – Leningrad. 56 pp. [In Russian].

Rubtsov I. A. (1956b). *Moshki (sem. Simuliidae): Fauna SSSR: Dvukrylye V. 6 No. 6* (Black flies (fam. Simuliidae): Fauna of the USSR: Diptera). AN SSSR, Moscow–Leningrad, 860 pp. [In Russian].

Service M. W. (1977). *Methods for sampling adult Simuliidae: With special reference to the Simulium damnosum Complex*. Centre for Overseas Pest Research, London, UK, 48 pp. ISBN 0-85135-087-9.

Sommerman K. M., Sailer R. I., & Esselbaugh C. O. (1955). Biology of Alaskan black flies (Simuliidae, Diptera). *Ecological monographs*, *25*(4), 345-385. DOI: 10.2307/1943307.

Usova Z. V. (1961). *Fauna moshek Karelii i Murmanskoi oblasti (Diptera, Simuliidae)* (The fauna of black flies in Karelia and the Murmansk region). AN SSSR, Moscow–Leningrad, 286 pp. [In Russian].

Yankovsky A. V. (1979). The dependence of the abundance of larvae and pupae of black flies (Simuliidae) in water bodies on the number of attacking females. *Parazitologiya*, *13*(1), 36-42 [In Russian].

УПУТСТВА ЗА УЗОРКОВАЊЕ И ПРЕПАРИРАЊЕ ЦРНИХ МУШИЦА (DIPTERA: SIMULIIDAE) – ПОКУШАЈ СТАНДАРДИЗАЦИЈЕ ТЕРЕНСКИХ И ЛАБОРАТОРИЈСКИХ ПРОЦЕДУРА

Александр Василев и Сергеј Аибулатов

Извод

Овај рад даје смернице за узорковање и препарирање црних мушица (Diptera, Simuliidae), са циљем стандардизације теренских и лабораторијских података. Аутори детаљно наводе методе обраде узорака црних мушица, од сакупљања до идентификације, за сваки стадијум. Такође, предлажу начин узорковања који узима у обзир различите факторе животне средине који утичу на бројност и распрострањење врста. Рад је заснован на личном искуству аутора и има за циљ да допринесе ширем разумевању екологије и таксономије црних мушица.

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