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Development of the Malaise trap: how to streamline the search for micro insects

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Insects are the most diverse group of animals on planet Earth, but our knowledge of them on a species level is quite limited. Entomologists have many tools in their arsenal to try to diminish the gap between knowledge and ignorance. One tool in particular is used to survey flying insects in large quantities in an area of interest. The Malaise trap designed by Réne Malaise is a tent like structure that passively collects insect samples and stores them inside a collector bottle for later inspection. The inspection process is laborious in any case, but if an entomologist is interested in the world of microscopic insects, the task is even more arduous. This thesis aimed to design a tool to ease the search for the smallest insects by combining the power of a Malaise trap with a passively sorting collector and to answer the question whether or not a malaise trap collector can be developed to sort insects? The tool was tested in the field in Turku, Finland, for a period in the summer of 2020 and the samples were stored in groups that represent the part of the collector they had been sorted to. Later, each individual insect's fore wing was measured. Insect were set in a class by the size of their fore wing and the probability of each member of a class to end up in the bottom part of the collector was calculated. Also, a comparative time trial study between the new sorting collector and a standard collector was performed to see if a temporal difference in insect hand sorting can be found. After analyzing the data, it was found that 92% of insects with fore wings smaller than 1 mm were sorted in a desired fashion and that there was no difference in the times elapsed during the time trial test. It was concluded that the tool works, but it should be used only after considering the possible pros and cons.

Key words: Malaise trap, Collector bottle, Micro insects, passive sorting

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Hyönteiset ovat maapallon monipuolisin eläinryhmä, mutta meidän lajitason ymmärryksemme niistä on edelleen hyvin rajallinen. Hyönteistutkijoilla on käytössään monipuolinen arsenaali työkaluja, joilla tiedon ja tietämättömyyden välistä kuilua koitetaan kuroa pienemmäksi. Yhtä näistä käytetään erityisesti lentävien hyönteisten tutkimiseen suurella näyte määrässä. Malaise-pyydys on Réne Malaisen kehittämä telttamainen rakennelma, joka kerää passiivisesti hyönteisnäytteitä keräyspulloon ja säilöö niitä myöhempää tarkastusta varten. Tarkastus on työlästä kaikissa mittasuhteissa, mutta erityisen vaivalloista silloin, kun hyönteistutkija haluaa perehtyä mikroskooppisten hyönteisten maailmaan. Tämä opinnäytetyö pyrki kehittämään työkalun, joka yhdistettynä Malaise-pyydyksen tehokkuuteen, helpottaa pienimpien hyönteisten löytämistä ja vastaa kysymykseen: voiko keräyspulloa kehittää hyönteisiä passiivisesti lajittelevaksi? Työkalua testattiin Suomen Turussa tiettynä kesän aikana. Hyönteisnäytteet talletettiin erillisiin ryhmiin niiden keräyspullon eri osista löytymisen mukaan ja myöhemmin jokaisen hyönteisen etusiiven pituus mitattiin. Hyönteiset asetettiin luokkiin niiden siipien pituuden mukaan ja todennäköisyys sille, että luokan jäsen päätyisi pullon alaosaan laskettiin. Uuden lajittelevan keräyspullon ja vanhan pullon välillä testattiin, löytyykö hyönteisten käsilajittelussa ajallista eroa. Datan analyysien jälkeen huomattiin, että 92 % hyönteisistä, joilla etusiivenpituus oli alle 1 mm, päätyi toivotulla tavalla keräyspullon alaosaan, ja että käsinlajitteluiden välillä ei ollut tilastollisesti merkitsevää ajallista eroa. Todettiin, että työkalu toimii, mutta sen käyttöönotossa tulee huomioida kaikki sen hyödyt ja haitat.

Avainsanat: Malaise-pyydys, keräyspullo, mikrohyönteiset, passiivinen lajittelu

Contents

1 Introduction

1.1 The Malaise trap

The Malaise trap was invented by Réne Malaise and the invention was first published in Entomologisk Tidskrift (1937). The trap is designed to intercept the flight paths of insects and to trap them in a container or a collector bottle. When insects are stopped by a Malaise trap, they usually climb up the mesh fabric until they reach the top and are captured by the collector filled with a killing agent. Towne's light weight Malaise trap is a very popular updated model that is used around the globe to passively collect samples of insect populations (Skvarla et al. 2021, Townes 1972). Townes trap works just as the original trap designed by Réne Malaise. In Malaise's 1937 article, he describes the advantages of the trap, of which the most important is the possibility to leave the trap unattended for a period of time. This of course saves time and trouble, especially in locations which are difficult to reach. The original design of the trap is very similar to modern ones, with the most obvious difference being the collector bottle. Originally it was called the cylinder and it had many compartments and shapes that create a form that insects cannot escape from. In it, a bottle of ethyl acetate is hung as the killing agent. The original collector is made of brass whereas modern ones are primarily plastic (Malaise 1937, Townes 1972, van Achterberg 2009). The collector nowadays is simpler with two compartments and the ethyl acetate is commonly replaced with a potent solution of ethanol. Ethanol has some advantages compared to dry killing methods: it works as a preservative that protects from decay as well as from other insects and the collector can be left unattended for longer periods of time (Skvarla et al. 2021, Townes 1972).

Different shapes of the trap have been developed in the hopes of increasing catch rates. Such models include the previously mentioned Townes model, Gressitt-model, Marston-model and many more, most of which have later been modified or refined by other scientists (Skvarla et al. 2021). Lots of development has gone into designing a Malaise trap with a lightweight build to help with transporting and assembling the actual trap, but little to none of development effort has gone to evolve the collector as more than a container for samples between examinations of the catch. Focus of developing the collector has been to increase its durability in different environments, its size, and the most effective angle for the collector to be hung up on. One type of collector for micro Lepidoptera is suggested by Upton and Mantle (2010) where a fine

metal mesh in a separate bottom collects micro Lepidoptera, but the literature only gives information about the emptying interval but gives no indication of how successful it is (Upton and Mantle 2010). There has also been an interest to create a collector that collects and arranges insects within specific time periods into smaller increments of the whole sample and to give temporal data from the sample such as the one proposed by Murchie et al. 2001 (see also Matthews and Matthews 1971, Townes 1972, van Achterberg 2009). It seems that there have been no attempts to make the collector a tool to help with the separation of smaller orders, so a study is indeed required. This thesis is particularly interested in micro insects i.e., microscopic insects.

An important factor for the effectiveness of the Malaise trap is its placement within the survey site. The trap loses its objectivity because it is very difficult to place the trap in a random location since a trap which is placed in a proper spot has more potential to gather insects than a mislocated one. The trap should be placed on a spot that is on a path for flying insects. A sunny edge of a forest with an opening in the shrub to create a funnel effect is preferrable. Other factors to take into consideration for a successful Malaise placement are landscape structure, precipitation, wind conditions and the orientation of the trap (Masner and Goulet 1981, Southwood and Henderson 2000, Skvarla et al. 2021, Sääksjärvi et al. 2004, Townes 1972)

1.2 Previous improvements to other parts of the trap

Insects that fly higher in the area of sampling need specialized traps. Many different methods for the capturing of insects with traps which are not on the ground have been suggested, such as a modified Townes type Malaise, which has been suspended between trees but the most prevalent and widely used is the Bugdorm SLAM (sea, land, and air Malaise). The SLAM, with a detachable bottom collector, combines the collecting power of a regular Malaise trap and bottom pan traps and it can be hoisted up to catch high flying insects, as advertised on the manufacturing company's website (Bugdorm.com 2021). Bottom pan traps resign at ground level of the trap and are designed to capture insects that fall off the netting with soap water or if the pans are colored, they act as lures for flower visiting insects (Epsky et al. 2008, mississippientomologicalmuseum.org 2022). When comparing the efficiency difference of a canopy trap to a classic Malaise trap, no clear conclusion can be drawn (Skvarla et al. 2021).

The traps have a good catch rate for large sized flying insects, but small-sized Hymenoptera (micro Hymenoptera) have been poorly represented due to many factors, which has prompted the development of traps that kill insects upon contact on the intercepting mesh wall. The need for such a modification might be due to a low phototropic reaction in the insects, which means that they are not inclined to climb up toward the sunlight on the trap. Another popular modification to the Malaise traps has been to equip previously mentioned pan traps at the foot of the mesh wall to collect specimen which do not climb their way to the collector. Multiple studies have shown that Malaise traps and pan traps differ in the volume of collected specimen and in the diversity of orders found in the traps. Pan traps might help in the capture of Coleoptera as well since they tend to drop themselves down when their flight is intercepted (Masner and Goulet 1981, Matthews and Matthews 1971, Skvarla et al. 2021).

Malaise samples consist of many different orders of insects but the most prevalent by far are Diptera and Hymenoptera, but also Lepidoptera are very often present and, if pan traps are used, Coleoptera can be found. The color of the trap can influence the sample size since different insects are drawn to particular colors and it is suggested that an all-black Malaise might go undetected by insects and so have less of a chance of being evaded (Matthews and Matthews 1971, Skvarla et al. 2021, Townes 1972).

1.3 How a Malaise sample is inspected

One of the most time-consuming tasks for an entomologist is the sorting of the collected samples. The number of different specimens in a single Malaise trap can be quite large and sorting them for research use requires a certain amount of expertise since a typical sample consists of thousands of individual specimens. Sorting is performed by inspecting the morphology of the specimen in the laboratory, but different bulk sample methods have been developed to identify specimen at the order level or lower with a barcode database. Such bulk sample identification still requires preparation work by hand under the microscope, including pre-sorting for tissue samples, to create order specific groups and this preparation work is still arduous and time-consuming Also, identification based on morphology is much less expensive. Otherwise, these barcode methods have potential to hasten the study of insects (Aagaard et al. 2017, Wang et al. 2018).

A relatively new method of creating data of an ecosystem's inhabitants is by taking samples of the environment and analysing them via DNA barcoding (Hebert et al. 2003). All animals leave a DNA trace: hair, skin cells, saliva, or excrements, and these can be found in soil or water samples. These samples are processed in the laboratory and the DNA is amplified with PCR, after which it is sequenced. The generated sequences are compared to pre-existing databases for matches. Metabarcoding techniques have huge potential in the field of ecology, since morphological identification expertise is dwindling among scientists (IUCN 2021). On the flip side, metabarcoding samples, which can be very large, are rendered quite uninformative if a match cannot be found in a database. Samples can still be uploaded to services that store them in hopes that the future would yield a match, but this requires identification of samples and brings us back to the root of the problem (Ritter et al. 2019, Taberlet et al. 2012). This problem in the system still gives plenty of room for morphological identification and the development of better ways of making the process faster and less tedious.

The identification process is started by removing the samples from the collector bottles and placing them upon a flat, high rimmed surface, from which insects may be removed one by one. Bigger insects have a tendency of grasping smaller ones upon death, so inspection must be done carefully under a microscope, so that all the insects can be examined individually. Inspected individuals should be catalogued in a manner that befits the study at hand and stored in ethanol (70%) inside marked jars or vials. A paper sheet with sample informationshould also be placed inside the container. Typically, all of this needs to be done multiple times so that all of the sample can be surveyed, since a single sample can contain thousands of specimens. In the process of collecting certain groups or insects of specific quality, the inspected and un-inspected samples need to be stored separately so as not to mix the two. If insects are measured, a microscope optic with measurement capabilities, a digital measuring system or a measuring tool under the petri dish should be used. For molecular studies, specimens should be captured and stored in 95% or above ethanol solution to make sure the DNA stays intact (Schauff 2001, Worthen and Jones 2006).

1.4 The inspiration for the development of a new type of collector

This thesis was sparked by the interest of ticks and more specifically, the castor bean tick *Ixodes ricinus (Linnaeus, 1758)* (Acari: Ixodidae). This small blood feeding disease vector has expanded its' range in Finland and it seems that the population is growing. In Finland there is a high interest for more information about *I. ricinus*, which can be seen in the abundance of participation in the Finnish crowdsourcing tick project (Laaksonen et al. 2017, Sormunen 2018). The interest stems from the fear of illness from a tick bite, since the Finnish *I. ricinus* population carries many agents of serious diseases, such as *Borrelia burgdorferi* (sensu lato) (Sormunen et al. 2016).

Controlling tick population growth is challenging. Well-tended lawns seem to have less ticks, but this benefit mainly manifests in urban and sub-urban areas, where lawncare is the norm (Klemola et al. 2019). *I. ricinus* can use a variety of hosts through its different life stages, such as rodents, birds, and deer. The control of these animals is a feasible option for reducing tick populations, but it is labor-intensive, and the effects are local – not to mention the ethical implications of thinning out a population of a host species (Stafford 2004). Biological control seems to be the most reasonable choice for keeping the tick population in check, and there happens to be a species that parasitizes specifically the castor bean tick and several other hard ticks: *Ixodiphagus hookeri (Howard 1908)* (Hymenoptera: Encyrtidae) (Hu 1998, Santos 2017, Sormunen et al. 2019). A single specimen of the species was captured from Seili island in the Archipelago Sea in Southwestern Finland in 2013. Also, a study by Sormunen et al. published in 2019 collected *I. ricinus* which where parasitized by *I. hookeri* in Seili without finding a single specimen of the parasite (Sormunen et al. 2019, Klemola et al. 2019). For this study, Ruissalo island in Turku was chosen as the study site, because it has groves that are in many ways similar to those in Seili. Likewise, tick densities are roughly equal in Seili and Ruissalo. It is important to find out if there is a native population of *I. hookeri* in Ruissalo, since the species seems to be quite adept at tuning its ecology to match that of the local populations of castor bean ticks. Therefore, introducing non-native strains as biological control might not do much to tame the tick population (Collatz et al. 2010).

Since Chalcidoidea – and to that extent Encyrtidae – are usually very small, (0,3mm-2mm) (Krogeros et al. 1990), they are not easily spotted on a Malaise sample if the trap is able to capture them in the first place since it might not always be the best option in surveying them. A new collector that passively separates the very small insects from the rest of the mass could be a useful tool in a study focused on micro

insects, and it could help free up hours of valuable time spent manually searching for these insects. It does seem that a trap using a vacuum to capture very small insects is presented by Hagler et al. (2002), but, although effective, it uses batteries that run out of power relatively fast and is not cost effective to be used by solar power. Also, this is not an improvement of the Malaise, which has proven to be an effective and popular tool. This thesis aims to show that new effective ideas can be devised from a single point of interest and explain why it is important to concentrate on a smaller increment in a large field of study. All this background information has led to these research questions:

(I) Can the Malaise trap collector be developed to sort insects to different size groups?
(II) Can the new collector be used to ease the search process for specific insects?
(III) Can a specimen of *I. hookeri* by chance be found in Ruissalo?

2 Methods

2.1 Designing and building the prototype bottle

The new design for the collector had to take a few factors into account:

1) It needs to be light weight
2) it must accommodate at least three levels of size dependent sorting
3) It needs to be simple to understand and reconstruct from cheap parts
4) It needs to be easy to use

An ecologist has a lot of instruments he needs to carry in order to perform scientific endeavors and therefore a new collector model can't add to that burden, so the material was chosen to be plastic, which is not only light weight, but durable and easily modified. Different metal constructs might also have been acceptable, but the building process would need specific tools, the cost would climb very high, and the weight of the contraption might have been a problem. The problem that was most difficult to overcome was to have a design that has different filters inside it. The problem was solved by cutting in half a standard collector bottle used by the University of Turku Biodiversity Unit and connecting it to a soda bottle. The soda bottle would be the bottom half, so that the standard collector side can easily be connected to previously used malaise traps and the soda bottles cap would act as a

valve, which is required for emptying the bottle. The bottles were connected with a tightening ring commonly used in plumbing, readily available in hardware stores.

The passive sorting is reliant on insects sinking, which happens inside the collector aided by gravity. Most dead insects sink in the ethanol and this downward movement is what powers the sorting. During the emptying, an opened valve also draws the alcohol out of the bottle and thus creates a downward force that drags floating insects downward towards the filters. Two different net- or sieve filters with differing mesh sizes were designed to be fit inside of the collector. The bigger sized mesh sieve, called the middle sieve, is at the connection sight of the two collector halves and has a mesh size of around 3mm by 3mm and was made of flexible plastic meant to be used as a vermin repellent around trees. It was hot glued to an old jam jar cover that was slightly grinded to fit snugly into the upper part. The lower net was mesh fabric bought at a hardware store with a mesh size of 1mm by 1mm.

All these parts are relatively easy to find and the whole apparatus can be built with hand tools commonly found at home, although hot glue and a sanding tool help with saving time and nerves. Every piece of the device was easy to obtain for a reasonable cost at a low effort and can be replaced with alternatives that are at hand.

Figure 1 The new collector: 1) The whole collector covered with masking tape to protect from the sun, 2) the middle sieve (3x3mm), 3) the top part with the middle sieve and net mesh connected, 4) bottom part showing the bottle cap used as a valve. Arrow: shows the direction that insects sink in the collector.

2.2 Field and laboratory work

2.2.1 Trapping

Two Townes type Malaise traps (Marris House Nets) where set in Ruissalo (Turku, Finland) on 27.07.2020: trap number 1 (60°26’12.5”N 22°10’24.8”E) and trap number 2 (60°26’11.4”N 22°10’30.5”E). Trapping continued until 18.8.2020. Both traps were equipped with the new prototype collector bottles, which were filled with 400-500 ml of ethanol (alcohol content 70%). Both bottles were replaced after one week of collecting and carefully, keeping the bottles top side up on a rack, transported to the laboratory to be emptied and inspected.

In the laboratory, the top bottle was opened and inspected for the first time. It was given an alcohol rinse to remove any dried insects stuck to the sides above the alcohol level. The removed insects were given approximately 10 min to sink inside the ethanol. Next the bottles were emptied by opening the valve on the bottom side of the bottle. The ethanol was slowly drawn from the bottle into a glass jar that had a mesh filter on top of it. This was done to hasten the collection of the smallest insect in the sample. After all the liquid had pored through the filter, the valve and the bottom half of the bottle were removed and inspected for insects which might have stuck to it and rinsed with alcohol to ensure all the insects where removed. The net (mesh size 1mm x 1mm) was detached and placed on a petri dish. The top half of the bottle was placed upon a petri dish to be disassembled later.

The filter was inspected, and all the insects were removed from it into a petri dish by using forceps and afterwards rinsing with ethanol. From the petri dish the insects were placed into a dated and named glass jar topped with ethanol to be stored for later classification. The top half of the bottle was disassembled by removing the middle sieve (mesh size around 3mm x 3mm) onto the petri dish. The top bottle was inspected for insects which if found, where removed by using forceps and an alcohol rinse. Insects on the middle sieve were removed by forceps and the component was inspected under a microscope to ensure all the samples have been collected. Insects from the top part were placed into different glass jar than the filtered insects. Lastly the net mesh was emptied onto a petri dish with help by forceps and afterwards inspected under a microscope. After inspection the insects were placed in a similar jar as all the previous samples and put into storage.

2.2.2 Identifying

The insects were held in storage until autumn of 2021, when it was discovered that almost all the samples from the first two weeks had been ruined due to dried up storage jars. The problem occurred for all of the first two weeks and 28.7-4.8. fabric mesh samples of trap number 1. As for the first two weeks of trap number two, only the jars containing fabric mesh and valve insects collected during 21.7.-28.7. were spared. Later these would also be inspected, identified by order and measured, but would not be used in the analysis. All other samples were fine and could be therefore used in the analyses.

2.2.3 Measuring

Fore wings were chosen as the measured anatomical structure since it remains straight in storage, has a pair for measurement if the other is damaged and it gives an indication of the insect's overall body size (Salcedo et al. 2019, Worthen and Jones 2006). The measuring occurred by using a measuring tool with an accuracy of 1mm beneath a petri dish and straightening the fore wings on the measurement area. The fore wings were measured from the tip of the wing to the thorax with the aid of a stereoscopic microscope and forceps. The length of the wing was recorded along with the insect's order, date of capture and the trap number. Encyrtidae were separated from the other insects after inspection to be looked at by a more trained eye later. All other insects were re-stored in their previous jars after inspection.

Because Coleoptera and Dermaptera fore wings are hard and curved along their dorsal side, they do not give an accurate measurement and were therefore chosen to be excluded from the study. Dermaptera also have very short fore wings and do not give an indication of their overall size. Other reasons for exclusion are if the wings are both badly damaged or if the insect's thorax is split or broken apart.

2.2.4 Comparative study

After every insect was measured, a comparative study was performed in the laboratory to see if the separation of micro Hymenoptera can be performed faster with the new collector than the standard model. The standard model was used as the control and the timed search was handled as if it was a regular Malaise sample. In the beginning of every test, 150 insects were chosen: 50 with fore wings larger than 4mm, 50 with fore wings sized between 1mm – 3,9mm and 50 micro Hymenoptera with wings smaller than 1mm. They were put into one of the collectors one by one, the collector was gently spun along its vertical axis and left to rest for 15 minutes after

which a timer was started. The timer was stopped after all 50 micro Hymenoptera were separated from the rest of the lot. This was repeated with the same insects on the other collector. A new sample was done with new insects and the order of the bottles used was switched.

3 Analysis

For the time tests,10 samples were timed in all and the number of micro Hymenoptera separated into the valve portion of the new collector was recorded. A paired t-test was performed for the times to see if a difference in time spend sorting is found.

For analyzing the fore wing sizes in relation to their placement in different parts of the collector, all insects were distributed into five different classes by their wing size, (A, B, C, D and E), where A contains all insects with wings smaller than 1 mm, B = 1 mm, C = 2 mm, D = 3 mm and class E contains insects with bigger wings than 3 mm. Every individual was set on a binary scale to determine the probability of an individual in a certain class being found from the bottom part of the collector: 1 = an insect was found in the bottom part of the collector and not in either of the mesh filters, 0 = an insect was not found in the bottom part of the collector. The probability that an insect was found from the bottom part of the collector was modelled with a generalized linear mixed model (GLMM) with binary error distribution and logit link function. Trap id (separate id for each trapping session and trap) was used as a random effect in the model.

4 Results

The difference in sorting time between the two bottles was tested with a paired t-test. The mean difference was 227.7 (so that standard bottle sorting took more time) and the 95% confidence interval for the mean was [-97.1, 552.5]. The difference was not statistically significant $t(df=9) = 1.59$, $p = 0.15$.

Table 1 Time comparison of the different bottles and the percent of <1 mm Hymenoptera sorted successfully by the new collector. The elapsed time is represented by seconds spent searching for <1 mm Hymenoptera and the success% depicts the percent of <1 mm Hymenoptera that were sorted through the valve.

	Time elapsed during inspection (s)		
Sample	New collector	Standard collector	Success %
1	1654	2016	100
2	1118	1602	98
3	623	1463	100
4	1023	1410	86
5	1640	2079	88
6	1775	1200	92
7	1001	1548	96
8	1261	1460	90
9	542	884	100
10	1466	902	94
Avg.	1210.3	1456.4	94.4

The mean probabilities [with 95% confidence intervals] for each class to be found from the bottom part of the collector were as follows: A = 0.924 [0.87-0.96], B = 0.687 [0.5-0.83], C = 0.182 [0.1-0.31], D = 0.032 [0.02-0.07], E = 0.015 [0.01-0.03] (GLMM, n=4553, $F_{4, 4548}$=279.08, p<.0001).

Table 2 The probabilities of each insect size class being sorted in the bottom part of the collector..

Class Least Squares Means							
Class	Standard Error	DF	t Value	Pr > |t|	Mean	Lower Mean	Upper Mean
A	0.2255	3.764	44906	0.0005	0.9246	0.8658	0.9588
B	0.1955	2.127	44624	0.0508	0.6875	0.4984	0.8296
C	0.2102	2.837	-7.12	0.0068	0.1829	0.1008	0.3087
D	0.3611	24.76	-9.40	<.0001	0.03250	0.01571	0.06604
E	0.3865	32.46	-10.72	<.0001	0.01562	0.007173	0.03368

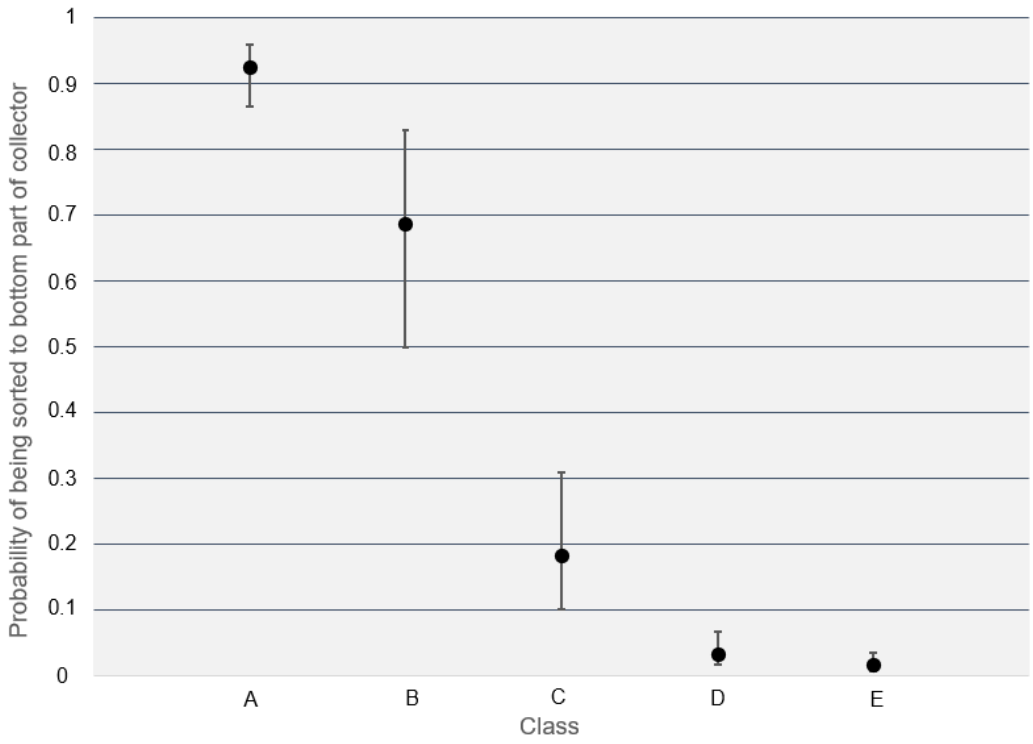

Figure 1. The probabilities of each insect size class being sorted in the bottom part of the collector

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The differences in the probabilities of each insect size class being sorted to the bottom part of the collector were statistically significant across all other groups (Table 3), except E vs. D [$t(4548) = 1.65$, $p = 0.0998$].

Table 2 Differences between different insect size classes in the probability of being sorted to the bottom part of the collector

Differences of Class Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer						
Class	**_Class**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
A	**B**	0.1328	4548	12.94	<.0001	<.0001
A	**C**	0.1531	4548	26.15	<.0001	<.0001
A	**D**	0.3312	4548	17.81	<.0001	<.0001
A	**E**	0.3587	4548	18.54	<.0001	<.0001
B	**C**	0.1038	4548	22.02	<.0001	<.0001
B	**D**	0.3115	4548	13.43	<.0001	<.0001
B	**E**	0.3404	4548	14.49	<.0001	<.0001
C	**D**	0.3203	4548	5.92	<.0001	<.0001
C	**E**	0.3485	4548	7.59	<.0001	<.0001
C	**E**	0.4557	4548	1.65	0.0998	0.4678

In order to determine whether *I. hookeri* could be found in Ruissalo, encyrtids that were separated were re-examined by this thesis' supervisor. No *I. hookeri* could be found.

5 Discussion

This thesis is quite unique compared to others done in the University of Turku in the field of ecology. Only one other publicly available thesis concentrating strictly on method evaluation could be found in the University of Turku utupub.fi search engine and even this was based on a previous study performed in Sweden (Jortikka 2020). There was no other thesis that was focused on a completely new method development, but it needs to be taken into consideration that the web based utupub does not hold every single thesis done in the University of Turku (utupub.fi 2022). It is hard to evaluate something that has no comparison, but there are findings for and against the new design and the supporting evidence and the following arguments should be evaluated by trained entomologists.

5.1 The results and analysis

The use for the new collector is somewhat specific and it should be used only after considering and calculating the possible temporal benefits. It can be used just like a standard collector to pre-separate different sized insects and storing them separately. An average 94% of the desired micro-Hymenoptera ended up coming through the valve in the laboratory tests, which is a similar percentage to the field tests (92%). It should be noted, however, that due to the different nature of the laboratory tests, direct comparisons should be made with caution.

While the collector works to a degree in its intended use, what about the loss of micro insects when discarding big insects? In this thesis, 92% of the insects with a wing size smaller than 1 mm sank into the bottom area of the collector, so 8% would have been lost if the collector would have been used as intended. A notable fact seen from the analysis performed with fore wing sizes as a class variable is that most of insects with a fore wing of 1 mm, class B, passed into the bottom part of the collector, which was not intended, and this might possibly be a clue that a smaller mesh could be implemented to further advance the separation of class A from the rest. A drastic drop of success is seen in class C, only 18% have passed into the bottom part and this indicates that the system works quite well by discriminating insects that have bigger fore wings than the size of the fabric mesh. More experiments are needed to evaluate the possibility of using a smaller mesh, but it might be that this could hinder the effectiveness of sorting the class A insects.

5.2 Problems and their reasons

The new collector is not without its fair share of problems. The time trials show that finding small insects even in small quantities is quite time consuming. If all the insects caught in the collector are stored, the time saved with the new method is decreased because it is not the intended use for the new collector. Malaise traps work quite well when searching for particular targets, but when taking only a few samples the ecology of a species cannot be determined (Evans 2016, Hopkins 2021). Optimally, the new collector should be used to only evaluate the diversity of a regions smallest insects by removing the need to comb every single Apidae hair for micro insects - in this the collector succeeds quite well. Because the new method works so well, it is possible discard the mass of bigger insects and concentrate on the smaller ones. On this note, it should be said that repeated samplings do not weaken the diversity of insects in a given area as concluded by Gezon et al. (2015). This is also linked to the fact that a Malaise trap has a limited area of effect, and most individuals are left at peace (Gezon et al. 2015, Gomez et al. 2018, Hopkins 2021, McCravy 2018)

At what range of percentile loss can the sampling be considered a success remains a mystery and this is an issue that needs to be considered by the ones using the collector. With the new collector, small insects can be removed from it in around 5 minutes, but it takes a trained person 30 minutes to go through a week's Malaise sample and this is when the focus is not centered around micro insects. Let's say a person is looking for micro-Hymenoptera, every bigger insect should go through a checkup under a microscope to make sure no small insects are stuck in their claws, teeth, or hairs. This naturally takes time and even then, there is the possibility that not all the wanted individuals are found. To get a thorough estimate of the time saved with the collector, it should be compared to a professional entomologist's ability to sort a whole Malaise sample by hand. However, due to time constrains, such a comparison could not be made for this thesis.

The reasons for loss of individuals in the samples are plenty for both the new collector and a standard collector, so a loss of individual insects is not only a problem for the new collector. When time is of the essence, the possibility to lose some small insects amidst other bigger ones most likely occurs quite often, especially if the survey is done simply by eye and not with the aid of a microscope. Sometimes insects get stuck inside the collector and need to be rinsed out if they are even noticed and during the pouring of alcohol into a petri dish or other similar surface, special care should be taken with the angle of the pour because it can splash over the edge and wash away samples with it. If working areas are cramped the risk of knocking down bottles, jars

and dishes is higher than when space is ample. Even when taking these precautions, insects can still be found lying on the table, stuck to jar lids, on the sides of forceps, clinging to gloves and clothing with no knowledge of how or when they got there. When small insects are given the possibility to dry in the absence of alcohol, they are very prone to being flung around the working area with even the slightest nudge. Some species of insects also have an almost transparent hue to their body, so even when it is thought that a petri dish is empty a closer look around the edges of the dish should be performed because some individuals might have evaded a researcher's eye multiple times. Other insects also create problems during inspection of samples as previously mentioned above, but Lepidoptera wing scales are a particular nuisance. They detach from wings and float on the surface of alcohol like tiny clouds and conceal some insects within them and as if that isn't bad enough the cloud follows every change in the surface tension and is drawn towards forceps during inspections. To help cope with these problems a way to simplify the system is a possible answer and the new collector does just that by removing need to inspect a part of the sample.

More ethyl alcohol had to be used inside the new collector so that evaporation wouldn't drop the alcohol levels below the middle sieve and the valve is simply a soda bottle cap. Because of the cap, upon release the flow was irregular and insects got stuck to the spirals of the cap. Also, it wasn't as intuitive to use as it could have been, but these problems could have been thwarted by using an actual valve. Sometimes insects managed to make their way between the middle sieve and the side of the bottle and were smashed broken. The biggest issue for the collector is that it can't be turned in any other direction ones it is in use. If the collector falls or is turned upside down the sorting process is disrupted and might no longer be viable. A specialized rack was created for transporting the collectors by car and this might be very hard to design around, and the only viable solution was to have the collector fit a mug holder inside a car, but this would have drastically shrunken its' size. A funnel was needed in the emptying of the collector, so this creates a need for more equipment and makes emptying the collector difficult but not impossible in the field. If such a task ever needed to be performed in the field the collector most likely needs to be disassembled altogether so that bigger insects can be removed, and this would be tedious even in the best of conditions.

5.3 Further improvements

There is still lots of room for improvement for the collector. The collector designed for this thesis was built of easy to find components from a hardware store and other parts, some of which were re-purposed trash found in a garage. Adhesive materials

and screws are an easy to find and use solution when making connections between the different parts and the rigid plastic mesh of the middle sieve could be replaced by fabric. It might even be that only one mesh net can perform the task of sorting effectively, but this has not been tested. Masking tape was used to protect the transparent bottom part from sun and this further show how a solution can be found quite easily for most engineering problems of the collector. With a little imagination the possibilities are endless. The design was originally intended to be done by 3D-printing that would have made the collector even more intuitive and could have been modified more easily to accommodate different needs, but a design that was chosen is symbolically made from accessible materials to show that anyone anywhere can create such a contraption. The result show that it works quite well for practically being a piece of trash.

The sampling time would possibly benefit from shorter intervals so that the upper parts wouldn't accumulate bigger insects as blockage. This would increase the number of times needed to empty the collector so it would negate the benefit of gained time and that is why it is important to optimize the use by evaluating how fast the bottle fills up and this of course is dependent on the region in which it is used and the placement of the Malaise trap. If all the issues above have been taken into consideration it is very important to store the samples correctly so that they will remain usable during the whole experiment. So, an understanding of insect trapping and studying is needed in any situation.

5.4 Benefits and possibilities

If a Malaise is used to sample the insect population of a given area, the use of the new collector is recommended if the focus of the study is the diversity of micro insects. The more the new collector is used, the more time is potentially saved.It should be noted though that these benefits are gained only if it is completely random which insects with fore wings shorter than 1 mm are left in the upper parts of the bottle. It should be studied if the new collector discriminates certain orders of micro insects and is unable to sort them in the way that is preferred.

Insects have been considered to indicate the whole of an ecosystem's diversity and state and we have barely scratched the surface of what more they could tell us (Brown 1997, Pakulnicka et al. 2015). As mentioned above in 1.4., some species can adapt to new environments, but it is important to understand why some areas contain only certain species and not others. Therefore, a full investigation of all species is required (Collatz et al. 2010). To manage such a task, the new collector could be implemented to be used just like a standard collector, by switching the focus from

small insects to bigger ones in different intervals. This is not the optimal way to use the new collector, but still allows for fast size-based assortment for the samples that can be stored for later inspection. It could be argued that even little passive sorting is better than none, but this requires further experimentation of the system in a larger setting and a focus on mapping an areas diversity of species. An advantage for the new collector is that there is no reason why it wouldn't be as effective in normal use as a standard one, since a Malaise trap is not particularly picky on what kind of a jar, bottle or cylinder is used to collect the sample, and if it can be fitted on the trap without changing its form by being too heavy, it should work. One more important consideration is that the bottle should be UV-protected so that it will not decay in the sun and so that the insects are protected (van Achterberg 2009).

If used correctly, the device could be a powerful tool to study micro insects because it is very specific in its' function. The specimens that are separated are barely distinguishable by the naked eye, so it is important to keep them separated from bigger individuals that might otherwise steal the limelight. The inspirer species *I. hookeri* shows that there is an interesting microscopic world with possibilities that can be of interest even to those with no scientific background and thus should be explored further. Perhaps the collector could be used in conversation efforts to hasten the search for endangered species to ensure that their habitats are not ruined by human endeavors. The interest should be among the scientific community as well, since metabarcoding might possibly revolutionize the field of ecology, but as mentioned before, it needs background information to work as intended and a DNA-library might benefit from tools that help it grow in diversity especially in the case of minute species that would otherwise be unobserved.

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