GONE WITH THE WIND? ALPINE WING ADAPTATION IN *KESSLERIA BURMANNI*: FLIGHT DYNAMICS AND SEXUAL DIMORPHISM

Submitted by

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Contribution

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Abstract

Insects` flight has been a topic of scientific study for centuries. The flight of Lepidoptera (butterflies and moths), the most diverse insect order on earth after beetles, has always been of specific interest. Comprehensive research on flight behavior, aerodynamics and wing structure has been done for butterflies, but only very little is known about moths. In Lepidoptera brachyptery, the anatomical reduction of wings, is very common in windy habitats and on islands and often causes reduced flight capability. Apart from walking, the effected animals use "jumping" as a form of movement. Females of *Kessleria burmanni* are brachypterious in that sense and expected not to use their wings during jumping. This work analyses the flight behavior of the Alpine moth *K. burmanni.* Methodologically it is done by using a wind tunnel and slow-motion tracking.

Male *K. burmanni* show a helical flight more likely to be short in time, but which can easily reach heights of about 40 centimeters (cm). This paper disproves the hypothesis that female *K. burmanni* do not use their wings while jumping. At room temperature (rt) a wing beat frequency of 35 Hertz (Hz) can be measured for the female moths as compared to 43 Hz in males.

Furthermore, microscopic and spectrometric analysis is used to discuss sexual dimorphism in terms of body color and wing morphology. In females brighter coloration and shorter wings are manifest, whereas the male moths are generally darker and have longer wings. Animals of both sexes display scales over the whole body.

Spectrometry analysis of the forewings of *K. burmanni* reveals extremely high remission in the ultraviolet spectrum. The maxima UV remission profiles in both, male and female moths, are closely spaced, indicating to be caused by some structural properties of the scales. Female moths exhibit higher remission than the males. During the process of scanning electron microscopy an irregularly structured lower lamina on the adwing side of the scales of a female moth is discovered for the first time.

Zusammenfassung

Seit Jahrhunderten untersucht man die Flugverhalten von Insekten. Lepidoptera (Schmetterlingen und Motten) sind nach den Käfern die artenreichste Insektenordnung. Ihr Flugverhalten ist schon lange von großem Interesse, deshalb wurden zahlreiche Studien zum Flugverhalten, zur Aerodynamik und zur Flügelstruktur von Schmetterlingen durchgeführt. Jedoch ist über Motten nur wenig bekannt. In windigen Habitaten und auf Inseln kommt es häufig zur Brachypterie in Lepidoptera. Sie wird als eine anatomische Reduktion der Flügel beschrieben, die oftmals zum Verlust des Flugvermögens führt. Betroffene Tiere bewegen sich folglich nur noch springend oder krabbelnd fort. Auch den weiblichen Tieren von *Kessleria burmanni* sagt man Brachypterie nach. Zudem, sollen ihre Flügel beim Springen gar nicht mehr zum Einsatz kommen. Wir haben das Flugverhalten der alpinen Motten *K. burmanni* mithilfe eines Windkanals und Zeitlupenaufnahmen analysiert.

In dieser Arbeit zeigen wir, dass der eher kurze Flug der Männchen von *K. burmanni*, bei dem sie jedoch auch leicht eine Höhe von 40 centimeter (cm) erreichen können, als helical beschrieben werden kann. Zudem wird von uns auch die Hypothese, dass Weibchen von *K. burmanni* beim Springen ihre Flügel nicht schlagen, widerlegt. Wir haben bei den weiblichen Individuen eine Flügelschlagfrequenz von 35 Hertz (Hz) beobachtet. Männchen weisen eine höhere Flügelschlagfrequenz von 43 Hz auf. Die Tiere flogen immer bei Raumtemperatur (rt).

Des Weiteren haben wir den Sexualdimorphismus, in Bezug auf Körperfarbe und Flügelmorphologie, mittels mikroskopischer und spektrometrischer Analysen aufgezeigt. Weibchen haben ein helleres Farbmuster und kürzere Flügel, während die männlichen Falter dunkler sind und längere Flügel haben. Tiere beider Geschlechter sind über den ganzen Körper beschuppt.

Die spektrometrischen Messungen der Vorderflügel zeigen eine extrem hohe Remission im ultravioletten Bereich. Die Maxima der UV-Remissionsprofile beider Geschlechter, liegen eng beieinander, was darauf hindeutet, dass ihr Ursprung in den strukturellen Eigenschaften der Schuppen liegt. Weibliche Falter zeigen zudem eine höhere Remission als männliche. Eine unregelmäßige Struktur auf der Schuppenunterseite einer weiblichen Motte wurde in rasterelektronenmikroskopischen Bildern entdeckt.

Introduction

With this work we want to contribute to the knowledge about *Kessleria burmanni,* and especially deliver new insights as to their manifold modes of adaptation to the rough climate conditions in Alpine habitats at high altitude. The following chapter will first be about insects in general and the fundamental principles of their ability to fly. Then we look at butterflies and their characteristic features, followed by a basic introduction to the genus *Kessleria* - including a short description of the phylogeny, the morphology of male/female individuals, their general behavior and their global distribution and habitat. Since animals and plants do have special possibilities to adapt to different living conditions this work will also provide a brief overview of the main methods of adaptation plants and insects have developed to survive in Alpine habitats and compare them with the findings from *Kessleria burmanni* in the laboratory (see Discussion).

What is an Insect?

There are approximately one million described insect species, but the real number is believed to be somewhat between 2.5 million and 10 million in total. Based on the correlation between insects and other organisms from the same habitat as well as their biomass, it is not surprising that insects are known to be the major category of animals inhabiting terrestrial ecosystems. Furthermore, they have invaded virtually every niche, including ocean shores and – in the singular case of *Halobates* - even the open ocean except the benthic zone (Grimaldi and Engel, 2005).

Insects are constructed after a segmental scheme. A hard and jointed exoskeleton formed by the cuticle, lending itself over the entire exterior body, is their most characteristic feature. It consists of a sequence of rigid plates, the sclerites, conjugated to one another by flexible membranes. Those membranes are also cuticular. Sometimes the sclerites are structured to allow accurate movement of one of the following parts.

Every segment of the insect corpus includes a primitive sclerite with dorsal orientation: the tergum. The tergum is again joined to a ventral sclerite, the sternum, by laterally membranous areas, called the pleura. There is also a jointed appendage originating from the sternopleural on each side. The segments are categorized in three units: Firstly, the caput (head), secondly, the thorax and finally, the abdomen. In the abdomen all kinds of the basic parts of the

segments may be greatly modified or even lost. The typical legs for walking are only kept on three thoracic segments. The cephalic appendages (head) are modified for sensory and feeding purposes. Usually the abdominal segments are for the most part lost, except some that may be altered into the genitalia, as for example in Apterygota, where certain pergenital appendages are maintained (Chapman and Chapman, 1998). Wings are also a useful specification and adaptation of this design, considerably improving mobility and thus dispersal and escape.

This unique design has preadapted insects for terrestrial existence in a wide variety of habitats and made them a very successful and diverse species all over the world. Judging from recent species and therefore a more recent innovation in insect evolution, holometabolous development has also fostered insects´ success and diversification. Today just four (Coleoptera, Diptera, Hymenoptera, and Lepidoptera) out of the overall 11 holometabolous insect orders contribute to about 80% of all insects. Typical of all of them is that they include a larva stage or even undergo a "complete" metamorphosis. Two of the four orders mentioned above contain the largest linages of plant feeding animals: The Lepidoptera (160,000 species) and the phytophagan beetles (100,000 species) from the Coleoptera (Grimaldi and Engel, 2005; Kristensen et al., 2007; Whiting, 2002).

About one million species of flying insects are known. Additionally, another 13 000 vertebrates, including mammals, some 9000 birds and 1000 bats are able to fly (Shyy et al., 2013). For more than a century, physicists and biologists have been fascinated by working on different forms or types of flight (Sane, 2003). For insects and their evolutional success, flight is the key innovation. Due to it they can disperse, migrate, show territorial as well as courtship behavior and avoid predators. Flight is highly diversified – there are even differences between some male and female insects as, for example, in butterflies. Whereas male butterflies tend to show a typical flight behavior in terms of territoriality and courtship, female ones display unique behavior when looking for host plants (DeVries et al., 2010).

As any type of movement, flight is based on the complexity between the flyer and its surroundings (Dickson et al., 2006). As known from various studies, the flight of insects is multi-faceted: Hovering, abrupt acceleration and rapid turn, all perfectly suited to their habitat, can be observed (Tanaka et al., 2005). In general, insects fly by flapping their wings, usually described as rhythmic and cyclic motion. By doing this, they create aerodynamic forces

to stay airborne as well as for forward and darting flight (Nakata and Liu, 2011; Senda et al.; Tanaka et al., 2005). As the size of the flyer is reduced – e.g. an eagle compared to a fruit fly – the wing-to-body mass ratio and the Reynolds number[1] tend to decrease, while the flapping frequency of the wings increases. All flyers need and use an integrated system composed of wings to create aerodynamic forces, muscles for the movement of the wings, and both sensory and controlling systems to guide and maneuver in the air (Shyy et al., 2016).

Features of butterflies

Butterflies and moths (Lepidoptera) are the most biodiverse order of all insects apart from beetles (Coleoptera) and presumably the group of invertebrates most studied so far (Goldsmith and Marec, 2009). According to the classification of Kristensen Lepidoptera manifest itself in 46 superfamilies, 126 families and 332 subfamilies with no less than 150,000 described species (Kükenthal, 2013; Regier et al., 2013). In the domain of the Alps estimations go up to some 5000 Lepidoptera species, 250 of which show strong diversification and endemic characteristics to their montane habitat (Huemer et al., 2016).

Even if they belong to the group of the simplest animals on earth Lepidoptera – beyond their beauty – serve important purposes: In their terrestrial habitats as major herbivores, pollinators of many plants and food source for other animals (Goldsmith and Marec, 2009; Regier et al., 2013). Lepidoptera also are of great interest in scientific research – insect conservation studies ("flagship" taxa), genetics, molecular biology, physiology, ecology, development and evolution – as they provide relevant model systems (Goldsmith and Marec, 2009; New, 1997; Regier et al., 2013). Reversely, however, many Lepidoptera can cause enormous damage as agricultural or forestry pests (Goldsmith and Marec, 2009).

The main features of Lepidoptera are the following: First, the presence of scaled wings, the morphology and function of which may vary not only between but sometimes also within species. Second, elongated mouthparts used for sucking (proboscis) and third, a holometabolous (complete) development of four stages (egg-larva-pupa-adult) (Goldsmith and Marec, 2009; Shreeve et al., 2009). The larval stage is often called "caterpillar" in the vernacular (Goldsmith and Marec, 2009).

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[1] The Reynolds number denotes the relationship between magnitudes of flow inertia and viscous effects

The genus *Kessleria* Nowicki, 1864

The phylogenetic position of *Kessleria* within the Lepidoptera

The first typus description of the species *Kessleria* was given by Nowicki in 1864. After years of false classification and many misunderstandings (phylogenetic position of several species, ecology, misinterpretation of faunistic data), a great deal of revision work including genital-morphological studies has finally led to a general clarification of the taxonomic circumstances of *Kessleria.* (Huemer and Tarmann, 1992). In the course and aftermath of this groundbreaking piece of research twelve new species, including *K. burmanni,* have been described (Huemer and Tarmann, 1992; Randl, 1993).

Kessleria is a genus of the European Lepidoptera of the family of Yponomeutidae first established by Stainton in 1854 (Huemer and Mutanen, 2015; Randl, 1993). Today a total number of 29 species is known in Europe only, 18 of which have been described in the last thirty years or so. There are two more species known in Asia and North America.

External morphology, studies of the genitalia and DNA barcodes have established the basis of the taxonomy of this genus (Huemer and Mutanen, 2015). Its phylogenetic position according to Huemer and Tarmann (1992) and Randl (1993) is:

Animalia (Linnaeus, 1758; Kingdom)
    Euarthropoda (Lankester, 1904; Phylum)
        Hexapoda (Latreille, 1825; Subphylum)
            Insecta (Linnaeus, 1758; Class)
                Pterygota (Lang, 1888; Subclass)
                    Lepidoptera (Linnaeus, 1758; Order)
                        Glossata (Fabricius, 1775; Suborder)
                            Yponomeutoidea (Kyrki; 1990; Superfamily)
                              Yponomeutidae (Stainton, 1854; Family)
                                Yponomeutinae (Kyrki, 1990; Subfamily)
                                  Kessleria (Nowicki, 1864; Genus)
                                    Kessleria burmanni (Huemer & Tarmann, 1992)

Morphological characteristics of *Kessleria burmanni* and a brief description of its general behavior

K. burmanni is white to greyish in color enabling them to perfectly adapt to their montane habitat. Individual color differences from various habitats may occur (Randl, 1993). In contrast to the males, the females are characterized by reduced body and wing size due to specific adaptations to rough climate conditions. Wing size reduction in females (brachyptery) is

typical for many families of Lepidoptera living on oceanic islands and in habitats with high winds (Huemer and Mutanen, 2015; Medeiros and Dudley, 2012; Randl, 1993; Sattler, 1991). Both sexes do have more or less white rings on their antennae (Randl, 1993). If weather allows, the adults of many Tyrolean *Kessleria* species like to sit on rocks near the host plant of their larvae. During intensive sunshine the moths hide in fissures of rock, under shady overhangs of rocks or once in a while under some parts of plants. Because of their minor ability to move the females remain mostly close to their birthplace awaiting the males for the copula. The copula takes place immediately after sunrise in the months of July and August. It is known that on this occasion the females show a saltatory, jumping-like locomotion, to reach the sparsely spread host plants where they lay their eggs. In the Yponomeutidae a colony formation is characteristic. The flight period of *Kessleria* lasts from the end of May to the beginning of September depending on the Alpine climate and maybe also correlating with the irregular growth of their larvae (Burmann, 1973; Randl, 1993). The characteristic feature of the larvae is their highly developed ability to spin communal webs. This, of course, gives a clue to the German name of the family: "Gespinstmotten" (Randl, 1993).

Global distribution of *Kessleria burmanni* and a description of their habitat

The family Yponomeutidae, including around 200 species, is spread on every continent, whereas the genus *Kessleria* is mostly found in the mountainous regions of the Palaeartic, especially in the Alps (Randl, 1993). The genus *Kessleria* is highly specialized to their montane habitat of the northern hemisphere. That is also why both, larvae and adults of the majority of *Kessleria* species, are very difficult to study in their habitat (Burmann, 1973; Huemer and Mutanen, 2015).

In the European Alps – evidence exists only from the Eastern Alps so far – they can be found at high altitudes of about 3000 meter (m) – existence, however, below 600 m is only sparse and exceptional (Huemer and Mutanen, 2015). Many of the places where *K. burmanni* have been detected, are difficult to find, quite inaccessible and very small in scale. There are some 10 sample sites in the Tyrol, which are located between 2000 m and 2900 m altitude (Randl, 1993).

The larvae of *Kessleria* species show a characteristic host plant limitation to Saxifragaceae and Celastraceae, which in return displays the strictly monophagous lifestyle of *Kessleria* species.

Most of the species feed on *Saxifraga*, two are restricted to the herbaceous Celastraceae genus *Parnassia* (Huemer and Mutanen, 2015). For *K. burmanni* only two host plants are scientifically proven: *Saxifraga caesia,* a lime, dolomite and amphibolite bound plant and *Saxifraga opppositifolia*, also colonizing on silicate rock.

The life of the animals and their habitat is shaped by the rough weather conditions of the Alpine climate, e.g. by a shortened vegetation period, night frosts, many freeze-thaw cycles in spring and autumn, a delayed disappearance of snow and ice on northerly exposed sites in spring, a higher degree of sun exposure and a higher risk of mechanical damage (Randl, 1993). All those factors indicate that a constant Alpine climate doesn´t exist and the microclimate, highly diverse and varying within short distances, must be expected to leave its imprints on the life and development of *K. burmanni* (Prohaska, 2011).

Adaptations to the Alpine climate
Plant adaptations
Alpine vegetation can be found everywhere around the globe, at all latitudes depending on altitude. Responding to the harsh and often unpredictable Alpine climate, plants have developed a variety of specialized features.

The most generally known and obvious adaptation is reduction in height, a tendency towards herbaceous lifestyle which has long been proven by science. Most of these herbs are perennials with large underground roots or a stem storage system, some plants are biennial and even fewer annual. Furthermore, trees only grow as twisted, shrubby (so called) "Krummholz" near the earth (Billings and Mooney, 1968; Körner, 2003; Stöcklin et al., 2009).

The most important specialization of Alpine plants may be found in their cell membranes, which allows them to tolerate dehydration to some extent. While the tissues of plants are freezing, tiny ice crystals are being formed in gaps between each cell, drawing water from protoplasts and leading to dehydration. Plants at high altitudes also must live with just about half of the partial pressure measured at sea level, additionally, carbon dioxide (CO_2) and oxygen (O_2) partial pressure are reduced proportionally, which further increases molecular diffusivity.

Also, the temperature is much lower in these regions - generally a decrease of around 1°C per 100 m in dry air conditions must be reckoned with, a fact which again lowers the rate of

molecular diffusion. Not to mention that the capacity of the atmosphere to take up moisture is extremely downscaled, even if this effect will only play out in case of total absence of convective transport, such as wind (Körner, 2003). On the contrary, high summer temperatures can strongly affect the cambial growth of the plants and their seed production (Billings and Mooney, 1968).

Plant life in an Alpine climate is challenged by a shorter vegetation period, more snow in general, the "longevity" of the snow and rather harsh conditions from weather-related extreme events (Stöcklin et al., 2009). Snow, however, is also responsible for some positive effects on plants: It isolates them against extreme temperatures, be it low temperatures in winter or high ones in the spring-sun before the soil has thawed.

In Alpine conditions live-sustaining strategies and metabolic resources are essential. Whereas evergreen plants break bud dormancy somewhat later, because their older leaves are capable of doing photosynthesis, local deciduous plants have to start producing new leaves as early as possible. Depending where the plant lives within the Alpine environment, also physiological adaptations are necessary for life under extreme conditions. Some plants have developed various kinds of dormancy mechanisms, including seed dormancy, seedling establishment, different triggers for germination (e.g. temperature or light), and fast forms of growth. Because sexual reproduction requires a lot of energy, many Alpine plants focus partially on vegetative reproduction. A reduction of pigments, such as chlorophyll, maybe above all be due to a proliferated UV_b damage and increases reflectance in some Alpine plants – a process which may play a vital role in the heat balance of the leaf.

Another important adaption has to do with the regulation of the water household. In Alpine habitats particularly, droughts during summer are very dangerous for some plant species. "Winter droughts", too, induce stress on the plants – they occur if the snow cover of the plant gets removed – e.g. by wind – and the plant starts photosynthesis while the sun is shining, but the soil still frozen. The plant then loses a decisive amount of water not compensable because of the frozen soil, the "drought" stress becoming even bigger in continuing wintery conditions. Therefore, in Alpine plants the development and regulation of a metabolic system to capture, store and use energy at low temperatures and in a limited period of time is the key to adapt to a harsh environment. Photosynthesis, metabolism and respiration are essential lifeguards for these plants (Billings and Mooney, 1968).

Insect adaptations

The environmental conditions and their repercussions on plants as described above do also have a tremendous impact on the animals living under such circumstances. Generally, such a harsh environment extremely reduces the number of total species (Sømme and Block, 1991). But just like plants, also insects have developed numerous adaptations to those conditions, allowing them to live in habitats of impressive altitude.

First, morphological adaptations are widespread and well known in Alpine arthropods: Wing atrophy, melanism for higher absorption of solar radiation, increased pubescence and several protections against heat loss. Also, a reduced body-size may be of advantage for finding shelter in microhabitats.

Second, physiological adaptations such as a higher resistance to dehydration, a higher tolerance against cold are very important for Alpine species. Proofs for a tolerance against freezing, hibernation and even supercooling, have been found by science especially in some Alpine beetle species. There is also evidence for some species being wholly enclosed in ice during winter, living in a complete absence of free oxygen (Anaerobiosis) proving an increased metabolic rate by the high concentration of lactate found in them.

Third, many behavioral adaptations of Alpine insects have been described. For regulating body temperature and keeping it many insects crave for warmer microhabitats or shelter under rocks or below the surface. Alpine butterflies for example, deliberately place the darker base of their wings vertically to the sun to heat up their bodies for flight. Also, nocturnal activity is favored by some species, maybe to maintain their water-balance and/or keep away from predators. In addition, many insects have a multi-annual life-cycle with two or more overwintering stages – some, however, live in a univoltine fashion (Sømme, 1989).

The aim of this study

This work wants to investigate and characterize the flight behavior of the Alpine moth *Kessleria burmanni* more precisely. The key focus is on the brachypterous or "jumping" females and their adaptations to the Alpine environment. As previous research in this field has led to the conclusion that flight must be impeded by the body-wing-ratio of female specimens and thus they can only perform some sort of jumping locomotion, our hypothesis was that they do use their wings for flight.

In an attempt unprecedented so far we cultivated *K. burmanni* under laboratory conditions. Our aim was to document the development from the larva to the adult moth, study typical behavior-patterns and analyze the movement of the animals exposed to strong wind. This paper hence examines the locomotion of *K. burmanni* in a self-made wind-tunnel including slow-motion-tracking. In the process of the study the flight of the male moths was also recorded.

The scales of the wings and their features were analyzed via transmission electron microscopy (TEM) or SEM, spectrometry as well as photographically under the microscope. We also did biometrical measurements of the *K. burmanni* specimens (n=105) of the Natural Science Collection of the "Tiroler Landesmuseen Betriebsgesellschaft m.b.H." to elucidate the dimensions of these insects.

Materials and Methods

Sampling

Larvae of *Kessleria burmanni* were collected at the circular path to the "Hafelekarspitze" and at the "Goetheweg" on the "Nordkette" mountains on June 15th and 26th and July 13th, 2018 respectively. The samplings at the "Blaser" were picked on July 12th and 18th, 2018.

Table 1: Sampling dates

Five sampling dates with sampling time, weather conditions, coordinates, participants and sampling area are shown.

Date	Time	Weather	Coordinates	Participants	Area
15.06.2018	09:00-12:00	5°C, chilly, alternately sunny and foggy, patches of snow mainly on slopes facing north	-	2	Nordkette: circular path to the "Hafelekarspitze" and at the "Goetheweg"
26.06.2018	10.30-15:30	13°C, cloudy, clear sight, cold, very few patches of snow (north slopes)	-	4	Nordkette: circular path to the "Hafelekarspitze" and at the "Goetheweg"
12.07.2018	09:45-12:00	17°C, alternately sunny and foggy, no snow	47°6.394'N 11°24.677'O (offset 4 m)	3	Blaser

13.07.2018	09:15-12:45	14,5°C, sunny, scattered clouds, no snow	47°18.986'N 11°24.291'O (offset 4 m)	2	Nordkette: "Goetheweg" and a slope to the west of the "Mandelspitze" exposed to the north-east (coordinates)
18.07.2018	09:28-10:18 10:25-11:15	13,2°C and 17,5°C, chilly and windy, scattered clouds, sunshine every now and then	47°6.398'N 11°24.685'O (offset 6 m) and 47°6.401'N 11°24.692'O (offset 4 m)	2	Blaser

The sampling areas described above were selected, because they fulfilled a combination of criteria: They were within easy reach, previous findings of larvae had already been attested and especially because they were strongly recommended via personal communication. Sampling at the "Nordkette" was carried out sporadically alongside the path, whereas at the "Blaser" it took place in a fixed 5 x 5 m square (**Figure 1 a and b**). *Saxifraga caesia* and if available *Saxifraga opppositifolia* were scanned for larvae of *K. burmanni* (**Figure 1 d and e**). The cushion plants were either dislocated with a knife or carefully examined on the ground by hand. Indication for larval existence was taken to be brownish leaves and the presence of communal webs to be seen with the naked eye. A plant, if "positively tested", was freed from clumps of soil and brought to the laboratory of Innsbruck University in a box. A plant without larvae was carefully replanted to its previous location.
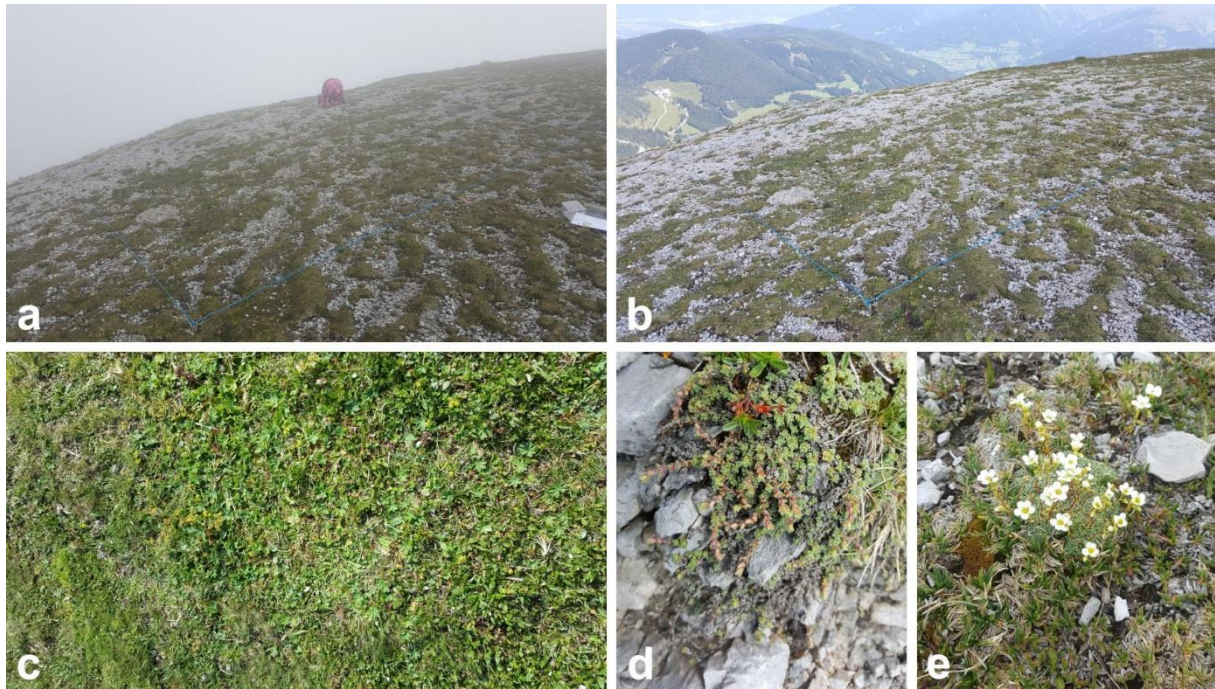
Figure 1: Sampling sites and host plants of Kessleria burmanni

(a) First sampling square at the Blaser; (b) second sampling square at the Blaser; (c) one sampling site at the Nordkette, a slope to the west of the "Mandelspitze", exposition north-east; (d) host plant *Saxifraga opppositifolia*; (e) host plant *Saxifraga caesia*

For means of comparison of data the main aim was to find the sampling sites as described and used by Randl (1993). Unfortunately, she had worked without any coordinates and so the location had to be estimated. At one previous sampling site, a slope to the west of the "Mandelspitze" at the Nordkette exposed to the north-east, sampling was not possible, because the former habitat of *K. burmanni* had been lost. No host plants could be found (**Figure 1 c**).

Sample numbers

A number of 26 larvae was collected on the five sampling dates. It is still possible, though, that not every available larva was actually collected, because many of them were too tiny to be seen or might have hidden themselves too well. The maximum in number was collected on July 12th 2018 at the Blaser, whereas on July 13th no larva at all could be found.

Table 2: Counted larvae on five sampling dates

Showing the number of larvae sampled on the different sampling dates. The highest number of larvae was found on the 12.07.208, whereas zero larvae were sampled on the 13.07.2018.

Date	Counted larvae
15.06.2018	1
26.06.2018	7
12.07.2018	15
13.07.2018	0
18.07.2018	3

Cultivating

After sampling, the cushion plants with larvae were transported to Innsbruck University in plastic boxes. There the plants again were freed from soil, put onto paper towels protecting them against mold and rearranged into various boxes so that each one was filled almost equally. The boxes then were labelled and stored in the cellar of the University near a wall to prevent it from heat. The paper was changed sporadically after some time to avoid moisture. Molded plants were continually removed.

From June the 13th caps of Eppendorf tubes filled with sugar water were put into the boxes but later removed, realizing that other insects got stuck in it, died and often water was spilled. However, to provide food for hatching moths, apple pieces were put into the boxes in Eppendorf tubes. Unexpectedly, those apples tended to become moldy within a very short time and also had to be removed. On the 04.08.2018 the larvae were transferred to fresh host plants via blunt-ended forceps and on the 17.08.2018 they were placed into another room with long-day conditions (16 hours) to prevent the larvae from falling into diapause. There the plants were moistened with water droplets at regular intervals and grapes instead of apples were inserted and exchanged once in a while. One additional transfer of larvae to fresh plants was carried out as well. Hatched moths were relocated to labelled petri dishes and stored in the crisper of the fridge. Half a grape was added to each petri dish. Both larvae and moths were frequently checked. Dead moths were weighed on an analytical scale and afterwards deep-frozen in an Eppendorf tube. To avoid sticking to the tube two weeks before the experiments the animals were transferred very quickly from the Eppendorf tube to a smaller petri dish (Ø 35 millimeter) with a fine brush. The petri dishes containing the thawing animals

were observed for approximately one hour to avoid insects come to life. After this process the dish was covered with a finely woven fishnet and stored in a drawer for drying.

Microscopy

Animals, both alive and dead, were observed and partly photographed through a Leica MZ8 (Leica, Germany) stereo microscope assisted by a mobile-phone-camera (Samsung Galaxy S6). The documentation of the dead moths and their scales was rendered by a stereo microscope model Leica MZ16 F equipped with a Leica DFC450 C camera. TEM images were taken on a Zeiss Libra 120 (Zeiss, Germany), whereas structural surface analysis was carried out with the Schottky Field Emissions Scanning Electron Microscope (2 kV, secondary electron and back scattered electron detectors) model Jeol JSM-7610F (Jeol, Japan) at the Material Technology Unit. The spectrometric analysis was accomplished under a Zeiss Axiovert 200 inverse light microscope with a Zeiss HAL 100 (12 V) illuminator. Images of the specimens from the Natural Science Collection used for biometrical measurements were generated with an Olympus SZX10 (Olympus, Japan) stereo microscope, a Panasonic Lumix DMC-GH4 camera (Panasonic, Japan) and Walimex pro LED Squares 170 BS (Walser, Germany) at the "Sammlung- und Forschungszentrum" of the "Tiroler Landesmuseen Betriebsgesellschaft m.b.H.".

Wind tunnel and slow-motion tracking

The wind tunnel was planned and constructed in analogy to the model of Nicola Rohrseitz and Steven N. Fry (2010). Its cubic dimensions of 150 x 40 x 40 cm, framed by Plexiglas of 1 cm thickness, allow transportation from one place to another (**Figure 2 a**). Due to the lack of a camera rack, however, slow-motion tracking was done with a Sony RX 100M5 (Sony, Japan) camera (aperture: 10; exposure time: $\frac{1}{2000}$ sec or $\frac{1}{3200}$ sec; ISO: 6400). To generate airflow, we used four 200 x 200 x 51 mm DC axial fans of the type 2218 F/2TDH4P by EBM-Papst (EBM-Papst, Germany). We also installed a 400 x 400 x 3 cm tubus core type PC (Tubus Bauer, Germany) with a tubular diameter of 3,5 mm to simulate the function of honeycombs for a laminar airflow (**Figure 2 b**). To provide the light for the slow-motion tracking, a 30 W battery-powered flood lamp (model Ritos type LY9603H, Ritter Leuchten, Germany) was used. The behavior of the moths was examined on different levels of wind velocity including lack of wind. Sometimes the animals had to be stimulated with the dull end of forceps to animate them to fly or jump.
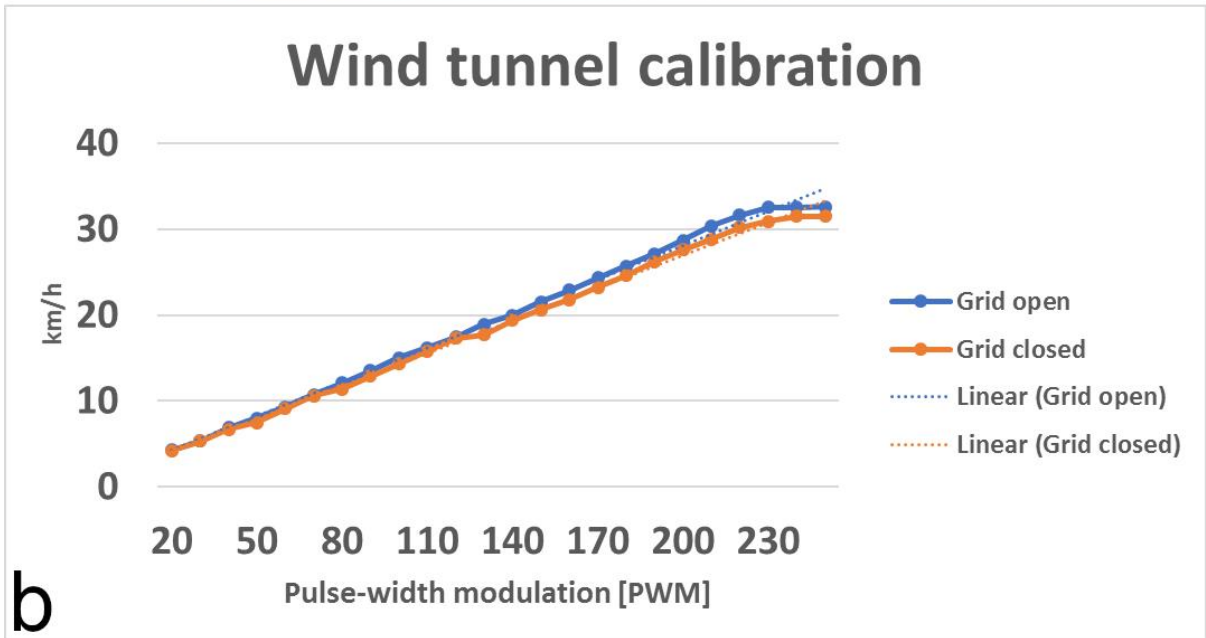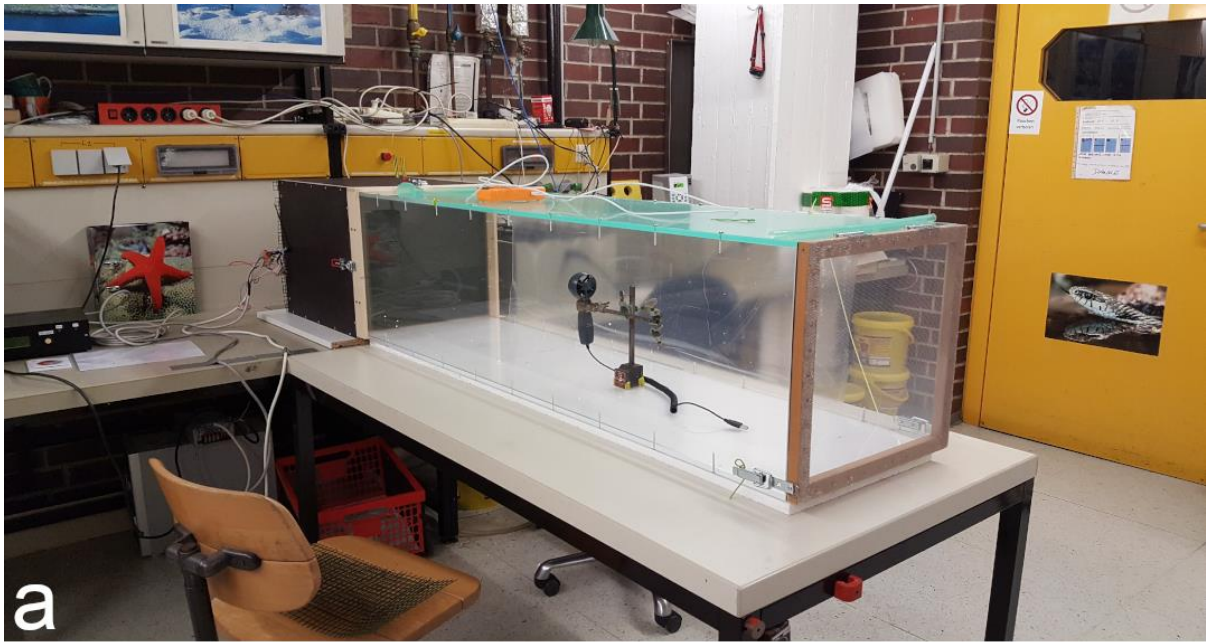
Figure 2: Wind tunnel and its calibration

(a) Wind tunnel in the laboratory; (b) calibration of the wind tunnel one time with the grid open (blue) and the second time with the grid closed (orange) with the pulse-width modulation (PWM) on the x-axis and velocity (km/h) on the y-axis. Both graphs show their maxima around 30 km/h and stagnate afterwards.

TEM embedding

Before embedding the samples, the epoxy resin (Epon, 100 %) had to be defrosted. In the meantime, small labelled sheets were placed in the mold. The chamber of the male wing sample (6) was tagged with 11v and the one of the female sample (4) with 12v. After defrosting the Epon was poured in the mold manually. After that, the samples were carefully transferred

to the resin and aligned by forceps. At this step some scales might have got lost despite utmost caution was being used. If existing, air bubbles were dissolved by hand. The mold then was left exposed to rt. for about one hour and later on moved to the compartment drier (60 °C) for curing. To avoid additional harm being done to more animals, the same moths came to be used as samples as the ones having already undergone spectrometric analysis. In them, the wings had already been plucked out.

SEM preparation

The following steps were carried out under the stereo microscope: Before proper preparation the sample was examined to locate the position of the scales and photographed under the stereo microscope. Then a double-sided adhesive carbon pad was fixed onto an aluminum specimen stub. The dried sample (Nr. 1) was gently squeezed with a cover slip and the remaining scales were transferred to the carbon tape with a fine brush. For our purposes it was absolutely necessary that the upper side of the scale be exposed.

Spectrometry

Spectrometric analysis was carried out with a Czerny-Turner monochromator with CCD component. Wavelength depending measurements were generated with an Ocean Optics USB2000-FL (Ocean Optics, USA) spectrometer, linked via optical fibers to the Zeiss Axiovert 200 inverse light microscope with a Zeiss HAL 100 (12 V) illuminator (Zobl, 2018). After testing a diffuse white standard and an already existing barium sulfate white standard – both being too big for the sample in terms of surface area – we decided to create our own white standard from barium sulfate. For this, we weighed a microscope slide on an analytical balance and afterwards put some barium sulfate powder onto it. We then exerted the pressure of a two kg mass onto the slide and then again measured its mass. To obtain the actual amount of barium sulfate, the two weights were subtracted from each other. In the end 0,038 g of barium sulfate was used as white standard for our samples. At the beginning and at the end of the process the barium sulfate was measured for calibration and also to see whether the light of the illuminator had degraded as time went on (**Figure 3 and 4**). However, at the end we measured at another position of the barium sulfate.

We adjusted the light beam to fit the relatively small sample of the investigation consisting of one forewing of an insect, carefully plucked out under the stereo microscope, put onto a microscope slide with the upper side up. We worked with two females and males each, and,

additionally, also measured the bottom side/adwing side or lower lamina of one female and one male forewing. We divided the wing in three parts, anterior/wing base, center and posterior/wing end, and measured the dimensions of every part three times (n=3 scans/measuring site). In reference to the paper of Vukusic et al. (2009) we also measured an aluminum foil.
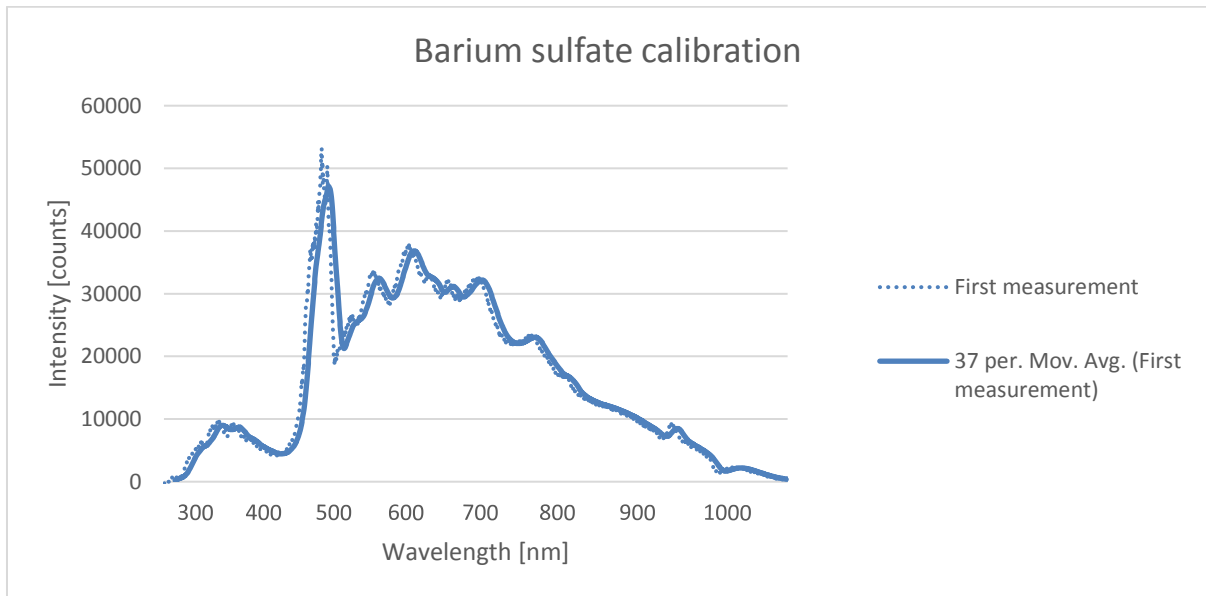

Figure 3: Barium sulfate calibration

Barium sulfate calibration with the wavelength in nanometer (nm) on the x-axis and the intensity (counts) on the y-axis. A moving average with a period of 37 was added as trendline. The maxima are around 53000 counts at a wavelength of about 420 nm.
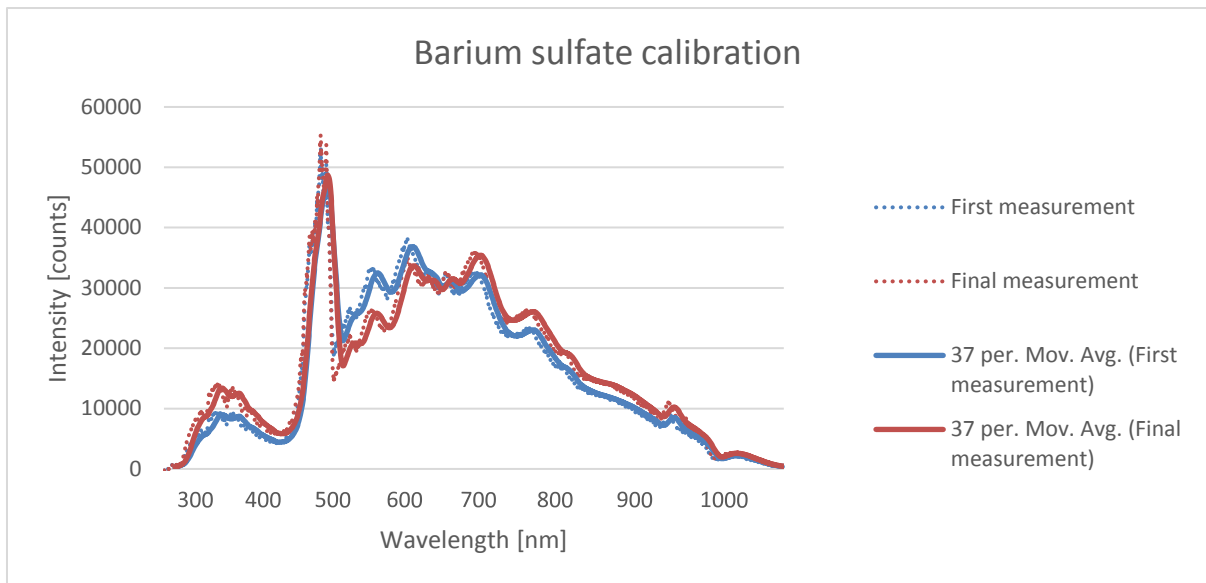
Figure 4: Comparison of barium sulfate measured at the beginning and at the end

Figure 4 shows the first and the last measurement of barium sulfate on two different positions. The wavelength (nm) is on the x-axis, whereas the intensity (counts) can be seen on the y-axis. Moving averages with a period of 37 were added as trendline. Both measurements have their peak around 53000 counts at around 420 nm.

Weather data

The weather data needed for this work were provided by the Central Institute for Meteorology and Geodynamics (ZAMG, www.zamg.at), the Department of the Tyrolean Government (avalanche warning service) and the Department of Atmospheric and Cryospheric Sciences at Innsbruck University (ACINN).

Software

Biometrical measurements and video cuts were carried out with the open source software Fiji (Schindelin et al., 2012), built on ImageJ v. 1.51d – v. 1.51s (Rueden et al., 2017). Images were edited with Gimp v. 2.8.22 (www.gimp.org). To visualize the spectrometric data, we worked with OceanView 1.5.2 (www.oceanoptics.com/product/oceanview). Most of the tables, the graphs and the calculations within the statistics (*average*-function and *stdev.p*-function) were rendered with Microsoft Office Excel 2016.

Results:

Life under laboratory conditions

This was the first attempt to cultivate *Kessleria burmanni* in our institute at the University of Innsbruck. With the help of Peter Huemer, we succeeded in documenting the development of eight animals from larva (**Figure 5 c and d**) to pupa (**Figure 5 e and f**) and finally, adult moth stage (**Figure 5 g and h**). Seven of these eight animals had been collected at the Blaser, one belonged to the first field trip at the Nordkette. Three hatched moths turned out to be males, the remaining five were females (**Table 3**). The longest life span in the laboratory was recorded for sample 2 with eight weeks and one day, whereas the average life span in the total sample amounted to 28,7 days (**Table 4**). Females on average weighed 2,203 milligram (mg) with a standard deviation (S. D.) of 0,325 mg. Male moths were found to be slightly lighter than their female counterparts with an average weight of 1,487 mg and a S. D. of 0,192 mg (**Table 5**). Sample 1, however, their weight should not be considered, because measurements were carried out a long time after the death of the animal, showing signs of complete dehydration.

Figure 5 Larvae in detail and adult moths of both sexes

a) Larva of *K. burmanni* in web on host plant; (b) larva outside web; (c) female *K. burmanni* (0 dph) sitting on vertical side of petri dish, exhibiting reflection of ventral side; (d) male *K.*

burmanni (0 dph) sitting on vertical side of petri dish. Larvae of (a) and (b) are according to Huemer and Tarmann (1992) 10-11 mm long. Moths in (c) and (d) are below 1 cm, though the male is bigger than the female moth. All specimens were sampled at the Blaser. Brightness and contrast adjustments were made. All images were taken with a mobile phone camera.
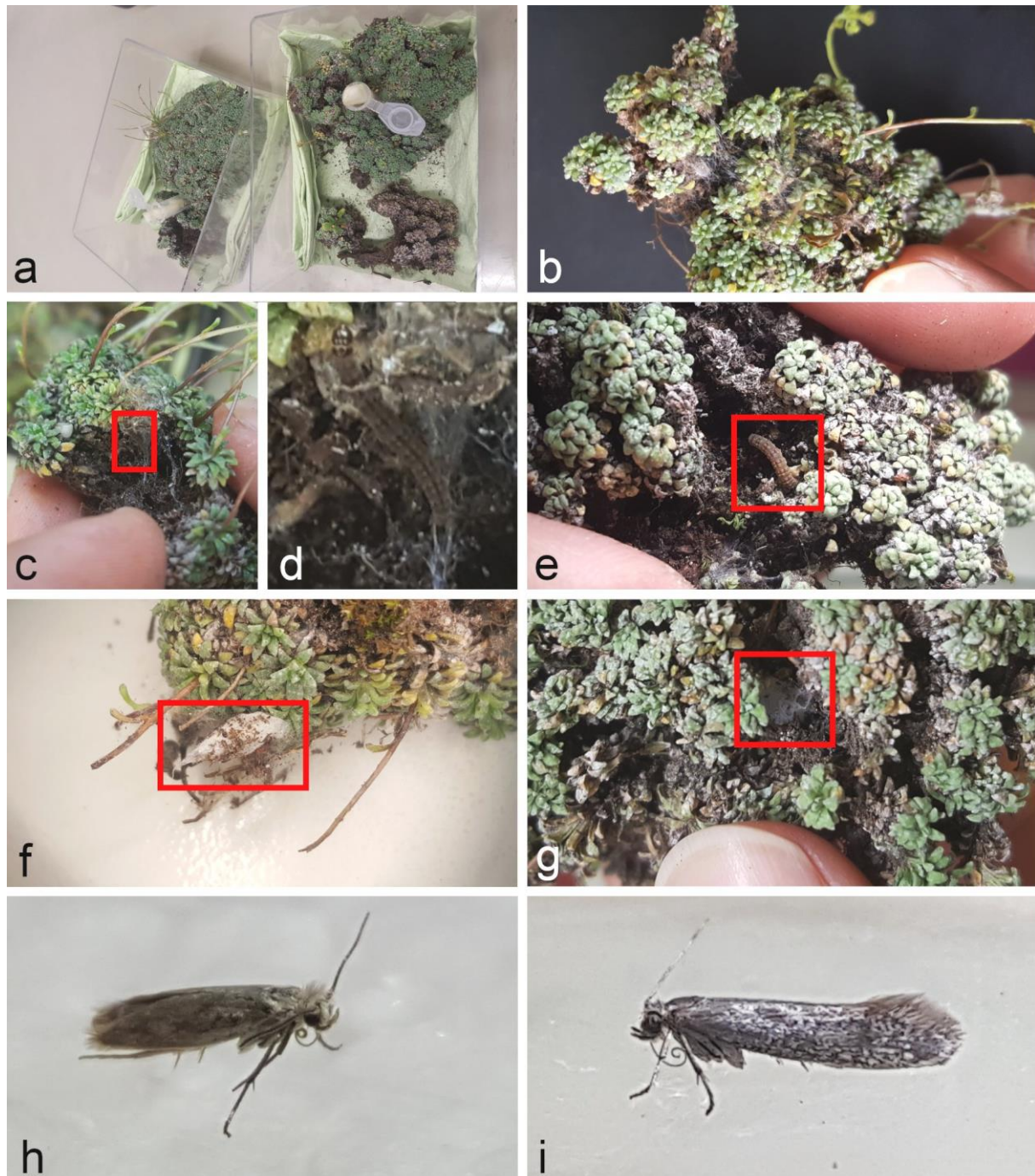

Figure 6: Development in laboratory conditions

a) Cultivating boxes with *Saxifraga caesia* and apple pieces in Eppendorf tubes; (b) host plant with webs of *K. burmanni* larvae; (c) larva between webs (frame); (d) framed larva (c) in detail; (e) another larva in detail (framed); (f) pupa in cocoon (framed); (g) abandoned cocoon of

already hatched moth (framed); (h) female moth (5 dph) viewed from the side – photo taken with mobile phone camera. Proboscis and spurs on the feet to be seen; (i) male moth (0 dph) from the side, photo taken by mobile phone camera. Proboscis and spurs on the feet to be seen. Larvae of (c), (d) and (e) are according to Huemer and Tarmann (1992) 10-11 mm long. Pupas under the cocoon in (f) and (g) are meanly 6,4 to 7,3 mm long (Randl, 1993). Moths (h) and (i) are below 1 cm, though males are bigger than female moths. All specimens were from the Blaser. Brightness and contrast adjustments were made.

Table 3: Sample data

The table shows the sampling date, the box and sampling location of the larvae, the hatching date, sex, the day of death and the weight of the moths as measured shortly before or after death.

Sample number	Sampling date	Box	Sampling Location	Hatching date	Sex	Day of Death	Weight (mg)
1	15.06.2018	2	Nordkette	unknown	female	10.07.2018	0,7 (weighed on 19.10.2018)
2	12.07.2018	4	Blaser	23.08.2018	female	19.10.2018	2,74
3	12.07.2018	1	Blaser	23.08.2018	female	25.09.2018	1,92
4	12.07.2018	4	Blaser	29.08.2018	female	11.09.2018	2,18
5	12.07.2018	4	Blaser	02.09.2018	female	21.10.2018	1,97
6	12.07.2018	4	Blaser	25.08.2018	male	20.09.2018	1,75
7	12.07.2018	1	Blaser	29.08.2018	male	11.09.2018	1,30
8	12.07.2018	1	Blaser	01.09.2018	male	18.09.2018	1,41

Table 4: Life span of the moths

The different life spans of the moths in our laboratory are shown. Sample 2 lived longest with 8 weeks and 1 day, whereas the samples 4 and 7 just lived for one week and six days. The life span of sample number 1 is unknown.

Sample	Life span moths
1	unknown
2	8 weeks and 1 day
3	4 weeks and 5 days
4	1 week and 6 days
5	7 weeks

6	3 weeks and 5 days
7	1 week and 6 days
8	2 weeks and 3 days

Table 5: Average weight with S. D. of our specimen of *Kessleria burmanni*

Showing the average weights of the samples with their S. D. categorized in total, male and female samples. Female specimens weighted 2,2 mg on average, whereas the male moths weighted meanly 1,5 mg.

	Total weight average (mg)	Standard deviation total	Female weight average (mg)	Standard deviation females	Male weight average (mg)	Standard deviation males
With sample 1	1,746	0,577	1,902	0,668	-	-
Without sample 1	1,896	0,449	2,203	0,325	1,487	0,192
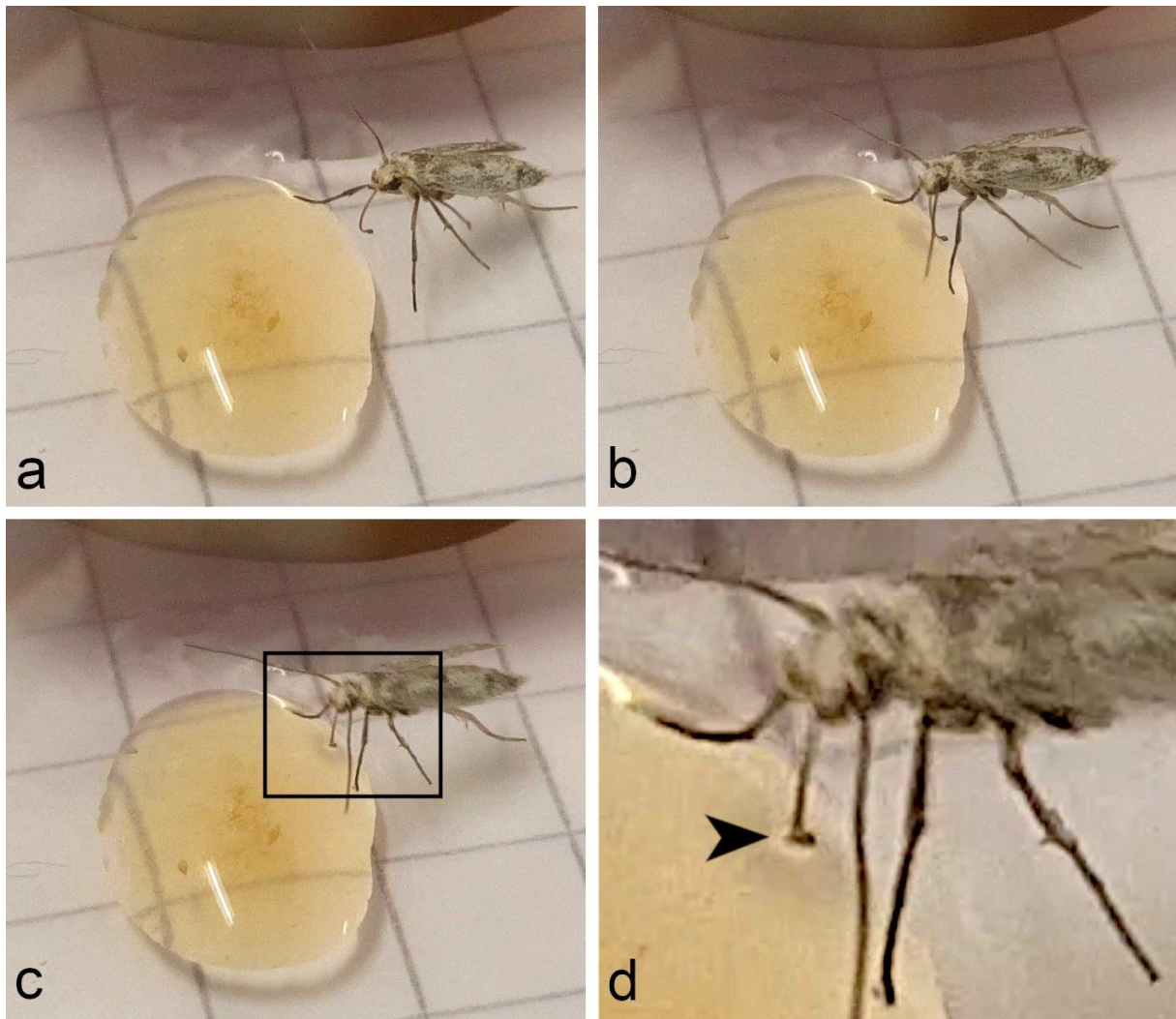
Figure 7: Female moth sucking on grape juice droplet

(a) Female moth of *K. burmanni* (9 dph), sampled at the Blaser*,* approaching the droplet; (b) adjustment of proboscis; (c) sucking; (d) section of framed part in (c) showing anterior of insect with proboscis (arrowhead). Brightness and contrast adjustments were made. Scale bars: quad paper, dimensions: 0,5 cm x 0,5 cm.

Feeding under laboratory conditions is crucial – this is the first study to report successful feeding of *Kessleria burmanni* in the lab. The moths suck on droplets of grape juice (**Figure 7**). The whole process only takes some seconds (approximately 2-5 sec) repeatedly over some time. The moths change target, approach the droplet, adjust their proboscis and start sucking. When feeding is finished the moths wrest themselves free from the droplet and move on to other business.

Movement of the moths

As known from other moths, *K. burmanni* is also attracted to light. The males are more inclined to fly when the flood lamp is on top of the wind tunnel than when placed sideways. The altitude of the spiral-shaped flight seems to correlate with the age of the animal – it is generally higher among the group of younger moths.

Although the moths can generally be said to be relatively uninterested in movement, after taking them out of the refrigerator they behave quite agile. However, older specimens are commonly less prone to move and they also struggle more with keeping their position when the wind in the tunnel is on. Also, no contact or interaction between the individuals, either in the wind tunnel or in a box, can be noticed.

During the experiments in the wind tunnel different patterns of movement could be seen. Generally, the insects move a lot more when the wind is off, in case of which they prefer walking to flying or jumping. Some moths remain in a kind of "parking position" when the wind gets too strong or they are stressed too much. Exposed to very strong wind they align themselves in the direction of the wind and "freeze" their movement with antennae and legs clinging to the body, the latter always kept in a tilted position. In addition, the animals seem to favor the vertical side of the glass in the wind tunnel as well as the vertical side of the petri dishes (**Figure 5 c and d**). Both, males and females, show interesting motion when lying on the back and trying to roll over. They do so by erecting their wings with the support of their antennae (**Figure 8**). After a failed jump or flight some similarly unique patterns of behavior are shown in order to roll over (**Figure 9**).
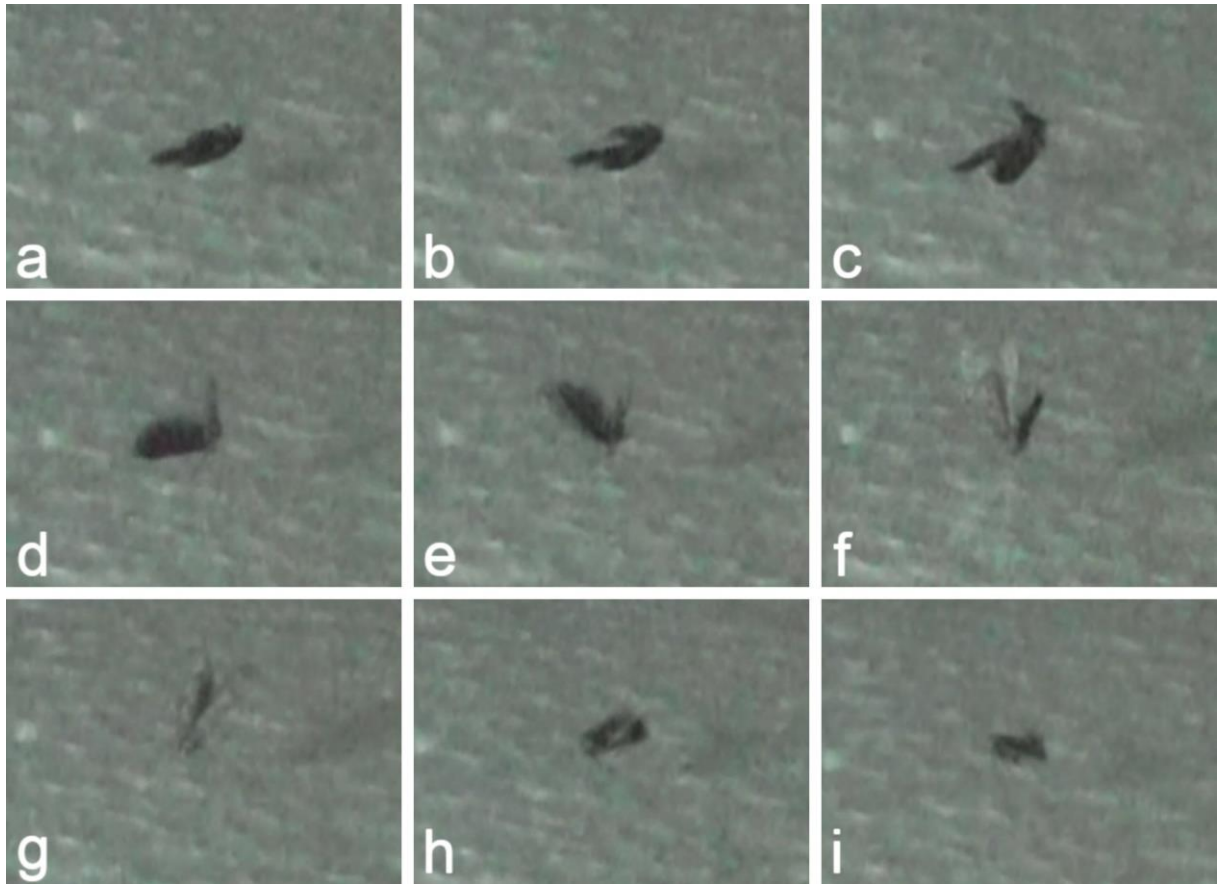
Figure 8: Turning over of a male moth

(a) Male moth of *Kessleria burmanni* (0 dph) from the Blaser, lying on its back with the abdomen pointing to the camera; (b) building up momentum and starting of erection of the wings; (c)fully erect wings; (d) vertical position of the moth with outstretched wings; (e-h) using momentum to turn fully around from the vertical position (d) to a seated position (h); (i) completely turned around moth in seating position with the head directed to the camera. All images were aligned that the head of the animal is positioned in the center of the image. The male moths´ length is below 1 cm. Brightness and contrast adjustments were made. Link: https://goo.gl/8aH6Mw
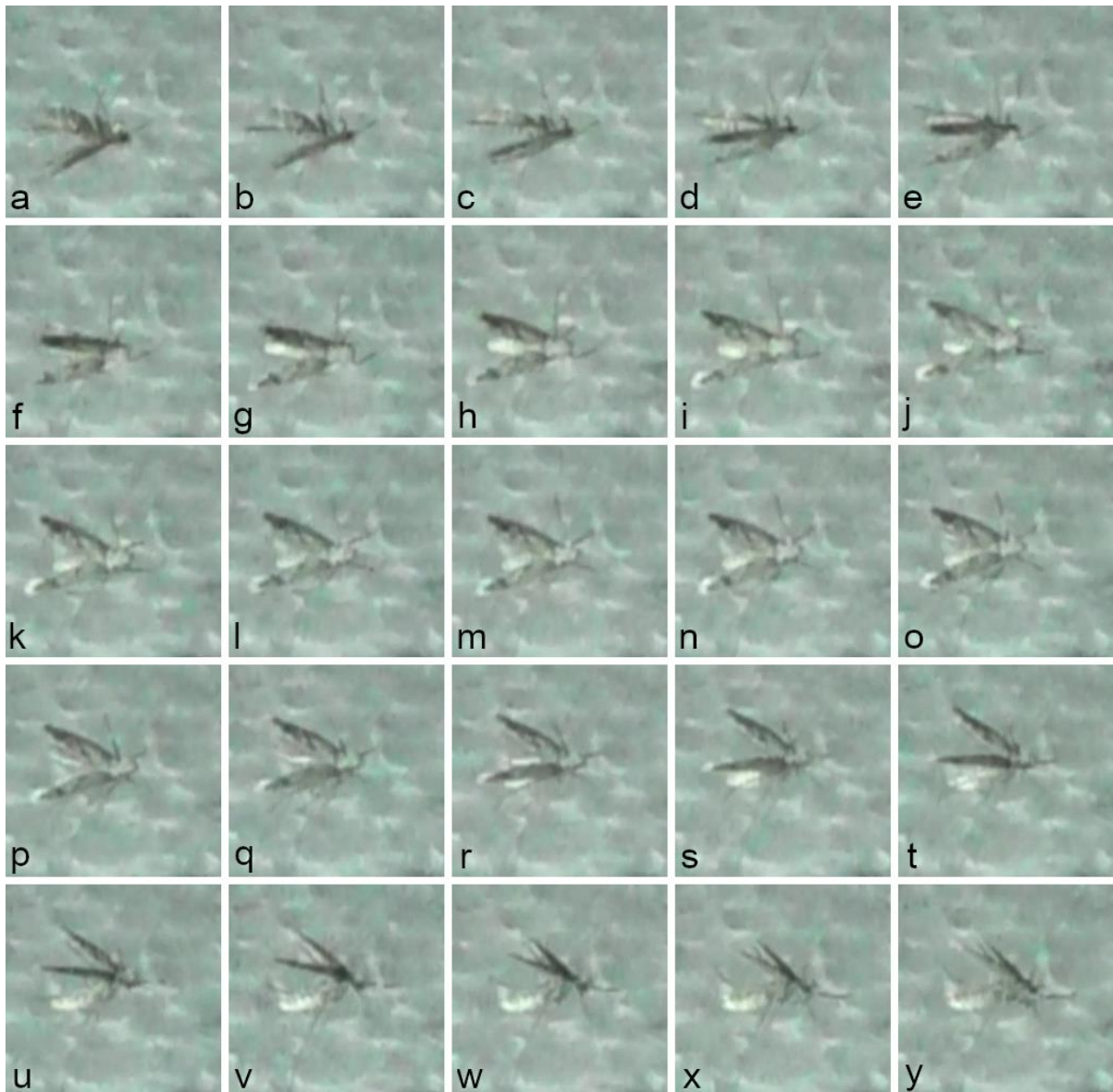
Figure 9: "Rolling over" by a female moth

(a-y) A female *K. burmanni* (0 dph), sampled at the Blaser, rolling herself over by using wings, legs and antennae, after a failed jump. (a-c) Building up momentum by dropping the abdomen top down; (d) lateral position of the moth; (e) adjustment of the antennae; (f-o) actual rotation of the animal, the "rolling over", by using the wings and antennae as support; (p-t) final part of the rotation realized only by the momentum and under support of the legs; (u-y) fully rotated moth getting into a seated position after rolling herself over. All images were aligned that the head of the animal is more or less positioned in the center of the image. The female moth is meanly 0,5 to 0,6 cm long. Brightness and contrast adjustments were made. Link: https://goo.gl/XvFCyS

Figure 10: Specimens of the Natural Science Collection in dorsal view

(a) Dried and preserved male *K. burmanni* sampled at the Muttekopf 1992 with parts of abdomen missing. Wings with long bristles and brownish appeareance; (b) another male specimen sampled at the Nordkette 1987 with one antenna missing. Forewings and backwings with long bristles. Forewings patterned with white and brown scales; (c) female *K. burmanni* from the Nordkette 1987. Forewings with little brown patches of pigmented scales; (d) another female moth sampled at the Daberklamm Süd 1988 exhibiting more brown patches in their forewings than (c). All images taken from the dorsal view. Scale bar: 0,25 mm.

As expected, the biometric measurements of the animals from the Natural Science Collection showed that the front wings of both, male and female moths, were longer and, thus, bigger in area than the back wings (**Figure 10, Table 6 and 7**). The area of the bristles, on the back wings varied from animal to animal depending on the preservation of the specimen (**Table 6**). As well as the area of the bristles, the phenotypes also varied depending on preservation and previous habitat of the moths (**Figure 10**). There is also a correlation between wing-length and habitat: The moths of the Juns-Alm (2300 m above sea level) are endowed with the largest front wings of a length of 0,670 cm on average and a S. D. of 0,282 cm. The largest back wings

can be found among the animals of the Landshuter Hütte at 2630 m. They have an average back wing length of 0,589 cm with a S. D. of 0,081 cm. By contrast, the smallest wings are those on individuals living at the Seehorn and Schneeberg Kaiserstein (both 2000 m) with the restriction that the number of specimens from there is very small (2 vs. 1 respectively) (**Table 8**).

Table 6: Average and S. D. of the total biometric measurements

The total averages and the S. D. of the front wing lengths, the backwing lengths, the front wing areas, the backwing areas and the backwing areas of the bristles are shown (n =105). Front wings are generally longer than the backwings and the front wing area is bigger than the area of the backwings. The area of the bristles on the back wings is nearly as big as the area of the front wings.

	Total average	**Standard deviation**
Front wing length (cm)	0,638	0,118
Back wing length (cm)	0,536	0,110
Front wing area (cm²)	0,073	0,024
Back wing area (cm²)	0,037	0,019
Back wing area bristles (cm²)	0,072	0,048

In *K. burmanni* the males are larger than the females. The front wings of the males are therefore 0,224 cm longer on average than those of the females and, thus, form a bigger area of about meanly 0,090 cm^2 with a S. D. of 0,011 cm^2. Also, the male back wings are about 0,214 cm longer, the area about 0.036 cm^2 bigger on average than in the female moths. Finally, also the area of the bristles on the back wings is obviously bigger in males than in females (**Table 7**).

Table 7: Sex depending averages with S. D. of biometric measurements of *K. burmanni*

Showing the averages with the S. D. of the front wing lengths, the backwing lengths, the front wing areas, the backwing areas and the backwing areas of the bristles in male (n = 63) and female moths (n = 42). Front wings, as well as the backwings, are generally longer and bigger in area in males than in female moths.

	Average females	Standard deviation	Average males	Standard deviation
Front wing length (cm)	0,503	0,044	0,727	0,044
Back wing length (cm)	0,405	0,031	0,619	0,036
Front wing area (cm²)	0,046	0,008	0,090	0,011
Back wing area (cm²)	0,015	0,002	0,051	0,007
Back wing area bristles (cm²)	0,014	0,006	0,109	0,015

Table 8: Total wing length averages with S. D. of front and backwings depending on the habitat of *Kessleria burmanni*

The averages with S. D. of the front wing lengths and the backwing lengths of all specimens according to their habitat are shown in this table (n = 105). The moths of the Juns-Alm (2300 m above sea level) have the largest front wings. The largest back wings can be found among the animals of the Landshuter Hütte at 2630 m. The smallest wings are those on individuals living at the Seehorn and Schneeberg Kaiserstein (both 2000 m).

	Front wing length total average (cm)	Standard deviation	Back wing length total average (cm)	Standard deviation
Muttekopf (2700 m) 1992	0,613	0,113	0,533	0,158
Nordkette (2300 m) 1970-1990	0,586	0,117	0,491	0,109
Blaser (2000-2241 m) 1989	0,645	0,096	0,545	0,085
Landshuter Hütte (2630 m) 1988	0,696	0,089	0,589	0,081
Juns-Alm (2300 m) 1989	0,670	0,282	0,565	0,095

Seehorn (2000 m) 1989	0,498	0,068	0,400	0,060
Daberklamm Süd (1520 m) 1988	0,584	0,119	0,495	0,108
Schneeberg Kaiserstein (2000 m) 1989	0,480	-	0,360	-

Close-up: *Kessleria burmanni* in detail

We investigated two specimens of each sex under the stereo microscope. There was a remarkably great difference in appearance between both sexes. The females looked brighter, somewhat cobby and with shorter wings, whereas the male moths made a darker impression in their coloration pattern and seemed to be slenderer with longer wings (**Figure 11 and 12**).
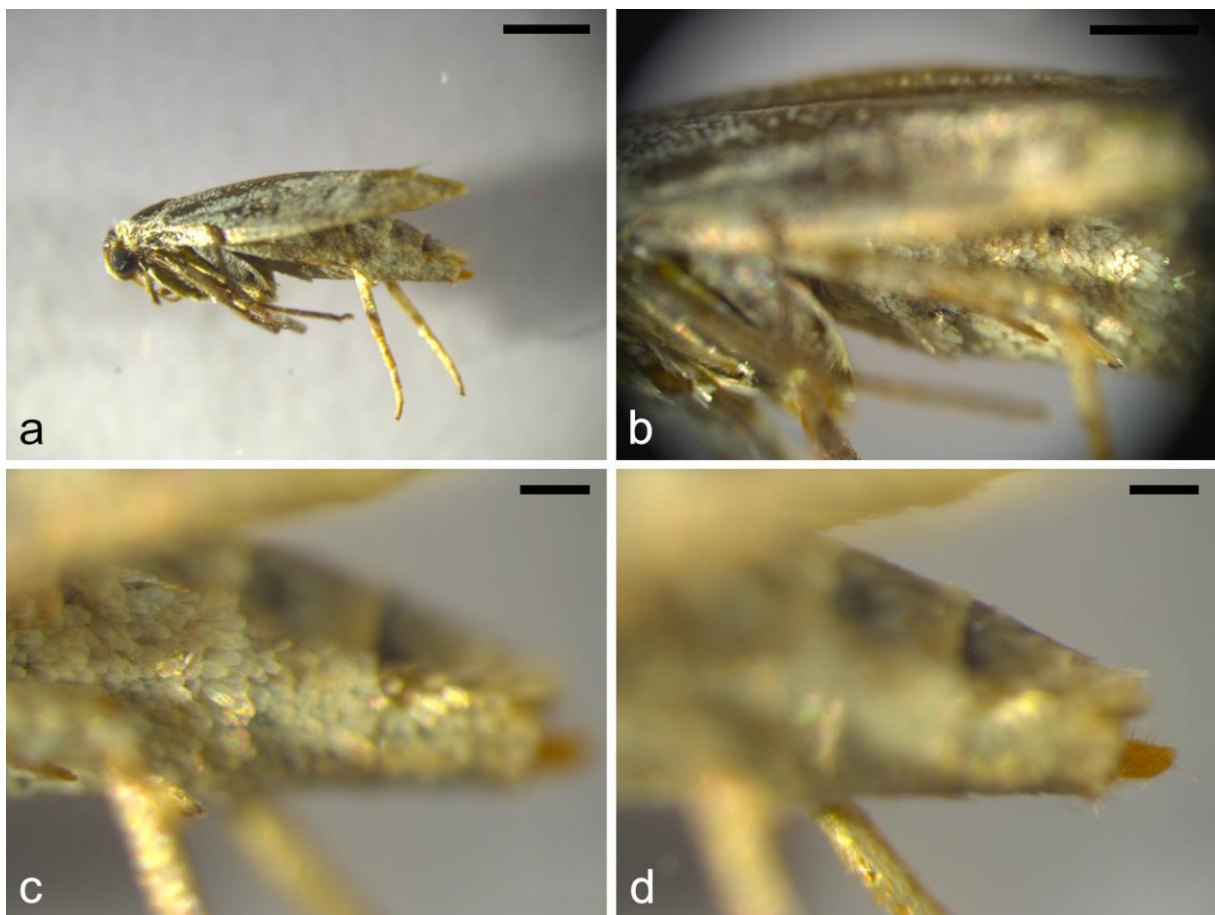

Figure 11: Overview of a female moth

(a) Dried specimen of female *Kessleria burmanni* from the Blaser, lying on the side under stereo microscope. Bare patches on wing, scales already lost; (b) detail of scaling of lateral

abdomen; (c) lateral view showing optical interference due to scales; (d) female reproductive organ from the side. Brightness and contrast adjustments were made. Scale bars: a and b: 1 mm, c and d: 200 µm.
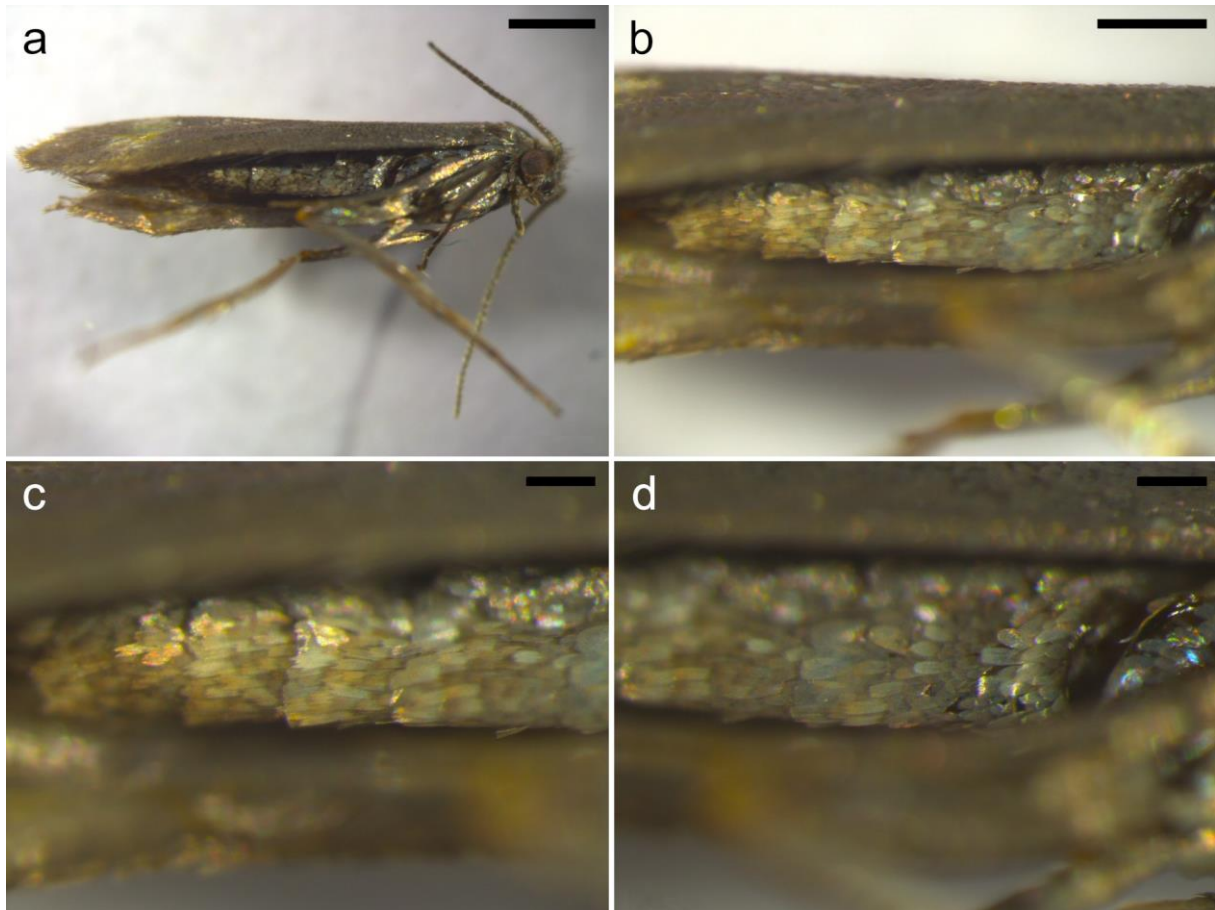

Figure 12: Overview of a male moth

(a) Dried specimen of male *Kessleria burmanni*, sampled on the Blaser, lying on the side under stereo microscope; (b) overview of scales of abdomen. Direction of head to the right; (c) Detail of scales of posterior abdomen seen from the side, head towards the right; (d) detailed view of mainly round scales of anterior abdomen from ventral side. Direction of head to the right. Brightness and contrast adjustments were made. Scale bars: a: 1 mm, b: 500 µm, c and d: 200 µm.

Table 9: Scaling in other dried Lepidoptera specimens

The scaling of the thorax/abdomen, the legs and the antennae of different dried samples of butterflies compared to *Kessleria burmanni* are shown in this table. *K. burmanni* and *Caligo memnon* show scales on every part of their bodies, whereas in *Papilio thoas* and *Aporia crataegi* the antennae are not scaled at all. *Morpho peleides* and *Cydalima perspectalis* showed scales on the thorax/abdomen and on the legs.

Scales	Caligo memnon	Papilio thoas	Morpho peleides	Cydalima perspectalis	Aporia crataegi	Kessleria burmanni
Thorax/Abdomen	positive	positive	positive	positive	positive	positive
Legs	positive	positive	positive	positive	no legs	positive
Antennae	only at the attachments	negative	no antennae	no antennae	negative	positive

Not only *Kessleria burmanni* but also other Lepidoptera exhibit full body scaling in varying degrees: Not only does *Caligo memnon* show scales on most parts of its body, legs and attachments to the antennae, it is also most similar to *K. burmanni* as far as patterns and outlook of scaling are concerned. On the other hand, in *Papilio thoas* and *Aporia crataegi* the antennae are not scaled at all and legs are missing. In *Morpho peleides* and *Cydalima perspectalis* the scaling of their bodies and legs was similar, but in both samples the antennae were lost (**Table 9**).

Different scale types

Despite their differences in outlook as seen under the microscope, males and females of *Kessleria burmanni* have one major feature in common: Their entire bodies are covered by scales, which make them appear like knights, suited in their best and shiniest armor. Because of reflections and interferences, it was difficult to obtain accurate images under the microscope.

Nevertheless, we were able to establish four different groups of scales according to shape in *K. burmanni* (**Figure 14, 15 and 16**): round, toothed, semi-round or semi-toothed scales (**Figure**

14 e, f and g) and the bristles (**Figure 15 e and f**). Each group of scales can again either be fully pigmented, pigmented on different levels or without any pigment at all (**Figure 14 h, i and j**). Furthermore, some of them show only structural colors as opposed to those with pigmentation in addition (Kilchoer Cédric et al., 2019).

No evidence could be found as to different positions of the scales in male and female specimens – generally it remains true that the males appear darker, which might indicate a higher degree of pigmentation of their scales. A categorization according to various parts of the body of *K. burmanni* likewise doesn't make any sense as the scales seem to be variously dispersed over the whole bodies of the animals (**Figure 13**). What is noticeable, though, is that the scales on the abdomen become rounder and bigger the closer to the thorax and smaller and more toothed on the hind part of the insects (**Figure 12 c and d**). On the wings, the scales are thinner and larger on the edges than in the center, the ones on the legs are bigger and longer near the thorax. There were too few scales left on the antennae to make any statement for this region (**Figure 13 b**).
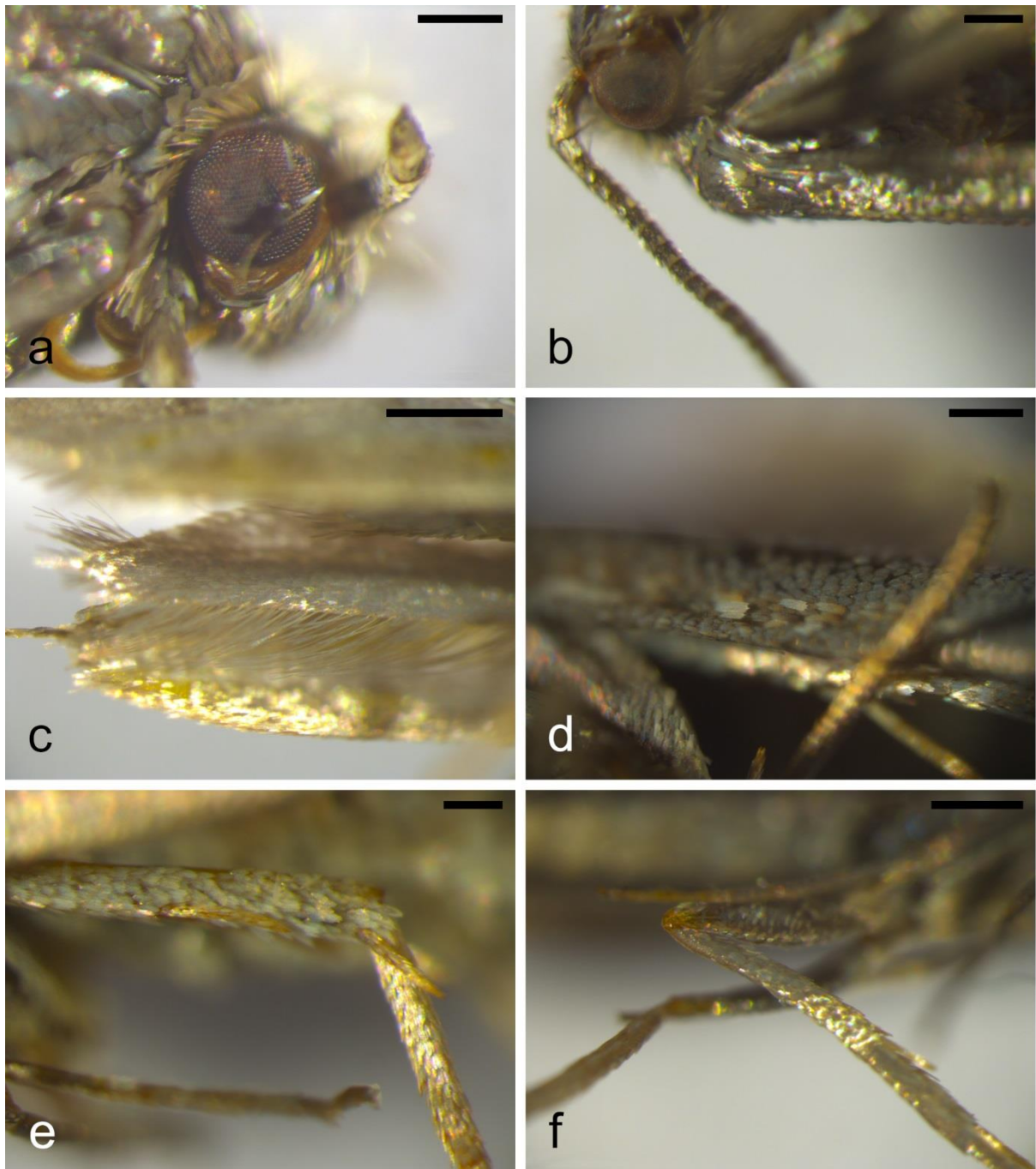
Figure 13: Whole body scaling

(a) Detail of scales on head of dried male moth of *Kessleria burmanni* proboscis and compound eye in center; (b) scaling of antenna of another male specimen of *Kessleria burmanni* and base of wings; (c) ventral side of wing with bristles of male moth of (a). Direction of head to the right; (d) scales of wing of male moth of (a), showing different pigmented scales and sizes. Direction of head to the right; (e) unpigmented scales on leg of female moth of *Kessleria burmanni*. Scales on the spurs; (f) legs of male specimen (a) with spurs. All specimens were sampled at the Blaser. Brightness and contrast adjustments were made. Scale bars: a and b: 200 µm, c and d: 500 µm, e: 200 µm and f: 500 µm.

Figure 14: Scales from the abdomen in overview and detail
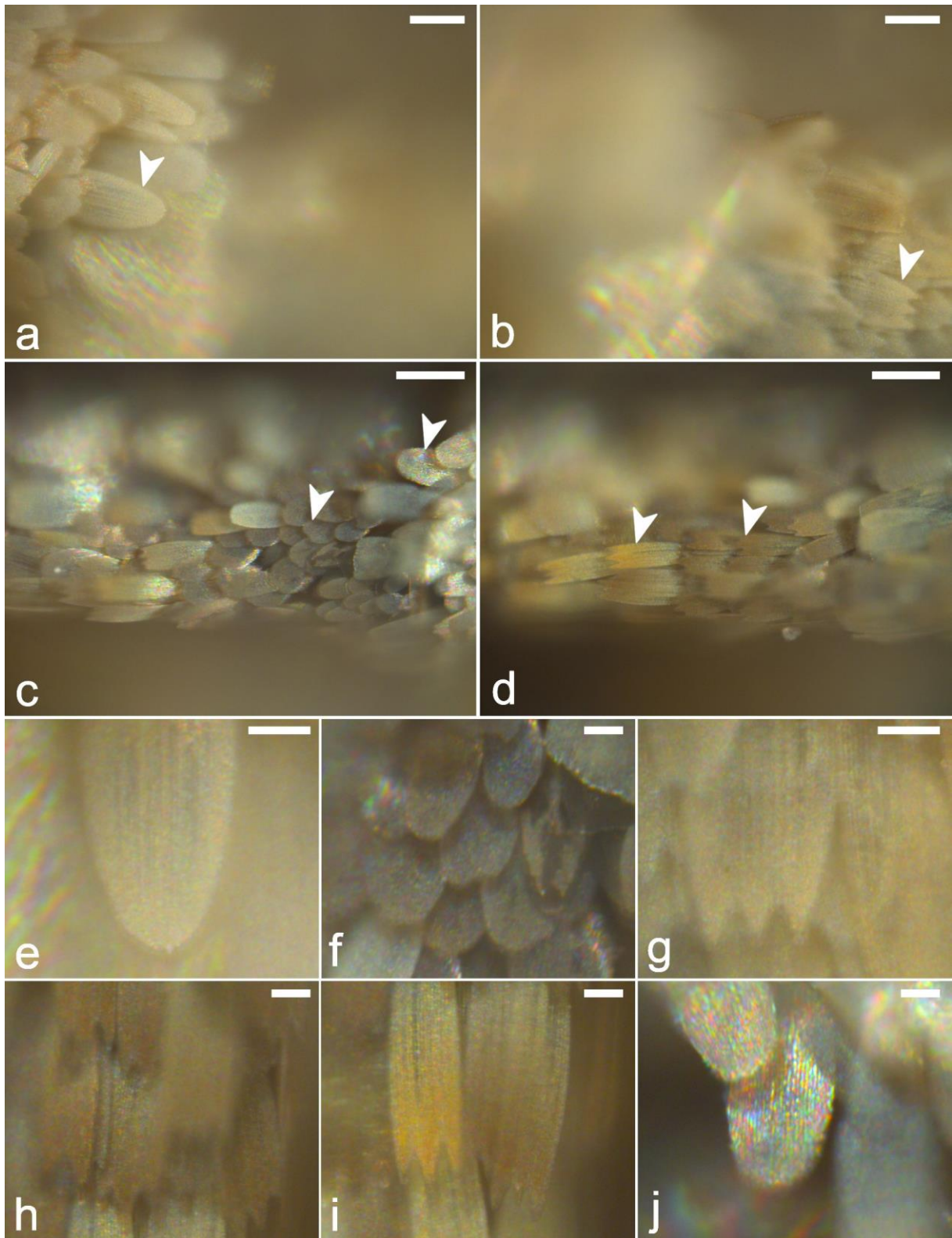
(a) Overview of different types of lateral abdomen scales in female moth of *Kessleria burmanni* with optical interferences. Head to the left. Image taken under stereo microscope, focus to the left. Arrowhead pointing towards round scale; (b) Second part of overview from (a) with focus to the left showing toothed scales (arrowhead); (c) Overview of various scaling of front

abdomen of male moth of *Kessleria burmanni*, head pointing to the right. Images taken ventrally with a stereo microscope. Arrowheads pointing towards semi-round (left) and round scale exhibiting structural colors (right); (d) Overview of scaling of the posterior abdomen of male moth with head towards the right-hand side. Images taken ventrally with a stereo microscope. Arrowheads pointing towards two toothed scales, one fully pigmented (right) one with lower pigment level (left); (e) round scale of (a) in detail; (f) semi-round scale of (c) in detail; (g) toothed scale of (b) in detail; (h) fully pigmented toothed scales of (d) in detail; (i) comparison of fully pigmented toothed scale (right) and toothed scale with lower pigmentation (left) of (d) in detail; (j) two round scales without pigmentation from (c) exhibiting structural colors. All specimens were sampled at the Blaser. Contrast spreading was performed. Scale bars: a and b: 100 μm, c and d: 200 μm, e, f, g, h, i and j: 40 μm.

SEM

The SEM images illustrate the surface of the four groups of scales mentioned above of a female moth, each as an overview and in detail. Two detailed images will be given of the irregularly structured lower lamina (adwing side) of a semi-round scale (**Figure 15 and 16)**. It is above all noticeable that no matter to which group the scale belongs the surface is always constructed in an identical fashion. It always contains the so-called primary ridges (Kaaber et al., 2009; Vukusic et al., 2009), longitudinal, parallel channels joined at intervals by smaller, concavely curved transversal channels, named crossribs (Zhang et al., 2015). Both together form a series of pores or channels that look like holes spread seemingly sporadically, which lead into the interior of the scale (**Figure 15 b, d and f**) (Ghiradella, 1998). In toothed scales the ridges in the curves between the tooth tend to become larger near the edges, which might be due to some aerodynamic features inherent in the scales. The name we invented for this phenomenon is "wing-jets" (**Figure 15 d**)

Table 10: Biometrical measurements of the surface structures of round and toothed scales and bristles

The averages and S. D. of three different nanostructures on round and toothed wing scales and on bristles in *Kessleria burmanni* are shown. The average gap between the primary parallel ridges is biggest in toothed scales and smallest in bristles. The same pattern repeats itself in the gaps between the transversal crossribs. Holes are meanly biggest in round scales and smallest in bristles.

	Average gap between p. parallel ridges (µm)	Standard deviation	Average gap between crossribs (µm)	Standard deviation	Average Ø holes (µm)	Standard deviation
Round scales	1,232	0,100	0,176	0,018	0,371	0,055
Toothed scales	1,638	0,239	0,218	0,013	0,287	0,037
Bristles	0,559	0,063	0,182	0,025	0,226	0,029

The biometrical measurements of three different scale types (round, toothed and bristles) showed the average gap between the primary parallel ridges to be biggest in toothed scales (1,638 µm - standard deviation 0,239 µm) and smallest in bristles (0,559 µm - standard deviation 0,063 µm). On the average the same pattern repeats itself in the gaps between the transversal crossribs. Here, too, the toothed scales also entail the biggest gaps (0,218 µm - standard deviation 0,013 µm) as opposed to the bristles (0,182 µm - standard deviation 0,025 µm). However, the average diameter (Ø) of the holes is biggest in round scales (0,371 µm - standard deviation 0,055 µm), medium in toothed scales (0,288 µm - standard deviation 0,037 µm), and smallest in bristles (0,226 µm - standard deviation 0,029 µm) (**Table 10**).
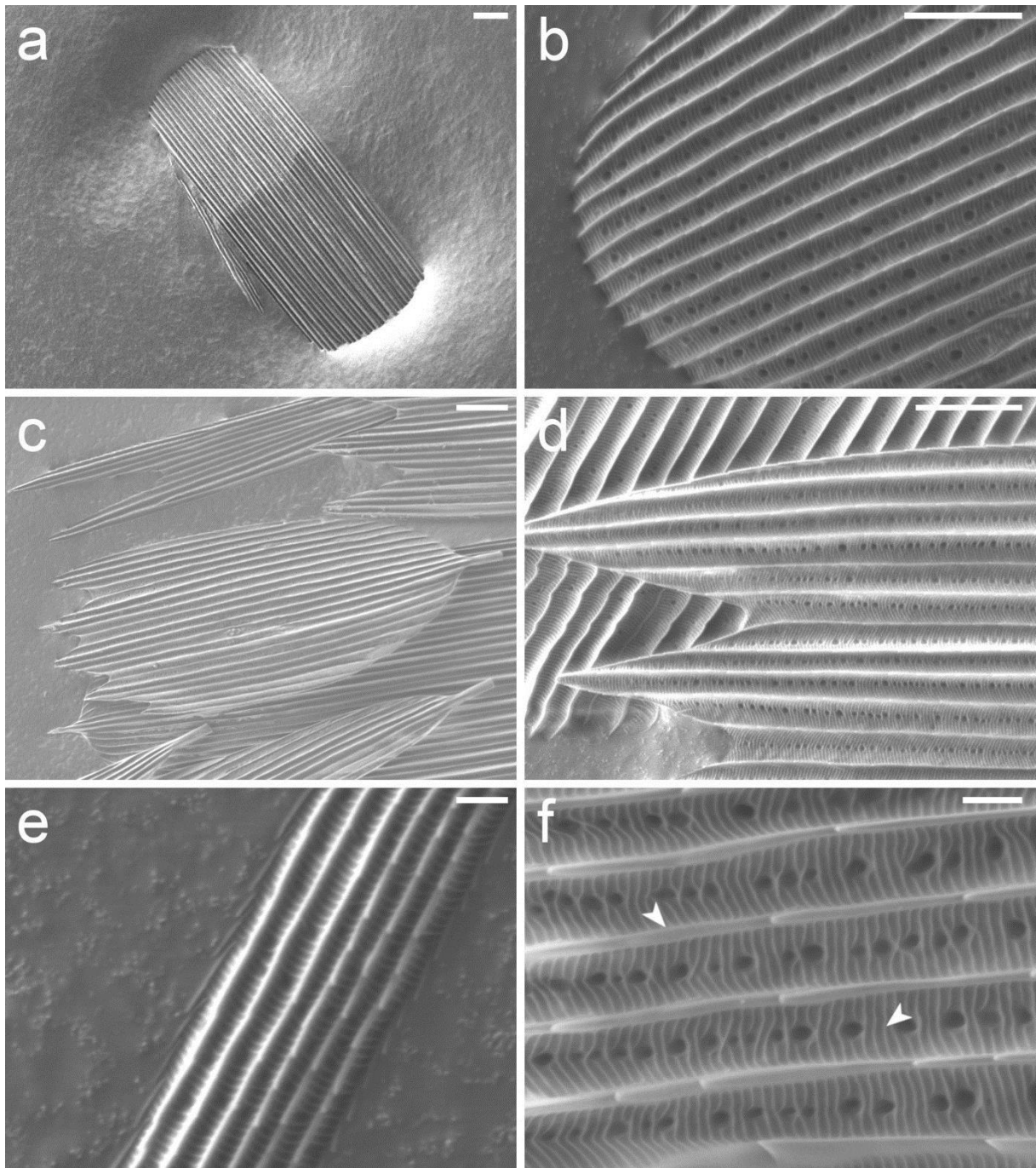
Figure 15: SEM images of round and toothed scales and bristles as an overview and in detail of a female specimen

(a) Overview of round scale partly broken off; (b) detail of surface of round scale with primary ridges, crossribs and holes; (c) overview of toothed scales; (d) detailed image of toothed scales. Wing jets at the edge of primary ridges in bends between tooths of scale; (e) overview of bristle part; (f) surface of bristle in detail. Arrowheads pointing towards primary ridge (left) and crossrib (right). All images were taken from the abwing side of the scales of a female *K. burmanni*, sampled at the Nordkette. Brightness and contrast adjustments were made. Scale bars: a: 10 µm, b: 5 µm, c and d: 10 µm, e and f: 1 µm.
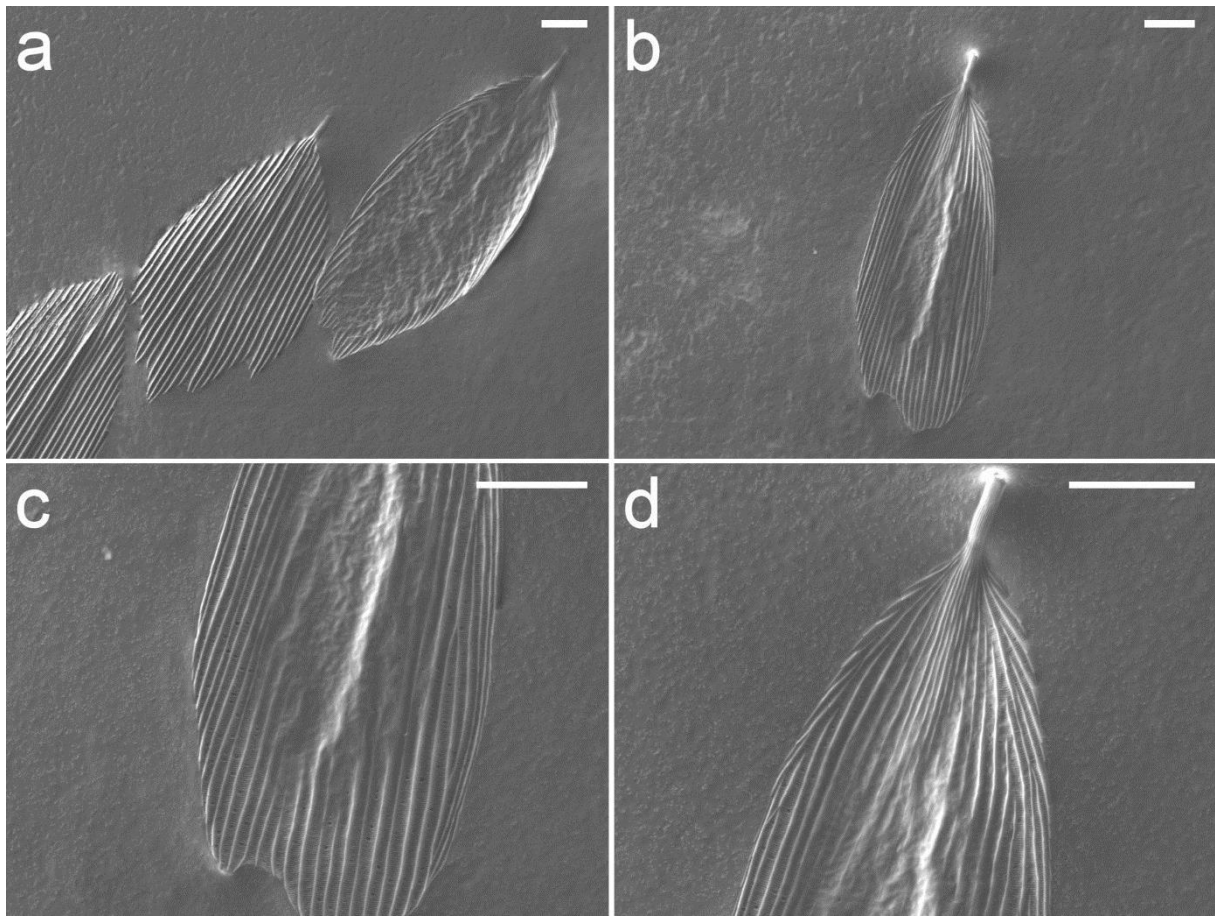
Figure 16: Comparison of upper side and bottom side of a toothed scale and a semi-round one of a female specimen

(a) Upper lamina (abwing side) of toothed scale (left) and irregularly structured lower lamina (adwing side) of semi-round scale (right); (b) overview depiction of semi-round scale bottom/adwing side up. Irregularly structured lower lamina; (c) detail of anterior of lower lamina of semi-round scale including primary ridges; (d) detail of posterior of irregularly structured lower lamina of semi-round scale with primary ridges. All images were taken from a female specimen of *K. burmanni*, sampled at the Nordkette. Brightness and contrast adjustments were made. Scale bars: a, b, c and d: 10 μm.

TEM

The following TEM images show three different scales from the last third of the left forewing of a male moth of *K. burmanni*. Nanostructures like the up-folded, parallel primary ridges, the concavely curved crossribs and the trabeculae are shown in cross section (**Figure 17 a**). In comparison to the biometric data of the SEM images of a female moth (**Table 10**), the gaps between the primary ridges in the male moth from the TEM images are nearly equal – with 0,923 μm on average. It is above all noticeable that the scales are pigmented in a

heterogeneous way – there occur even differences in one and the same scale -, where most of the times the cover scales exhibit a higher pigmentation than the ground scales. An irregularly structured lower lamina (adwing side) is predominantly to be found on the edges of the scales, which can also be noticed in the SEM images of the female moth of *Kessleria burmanni*.
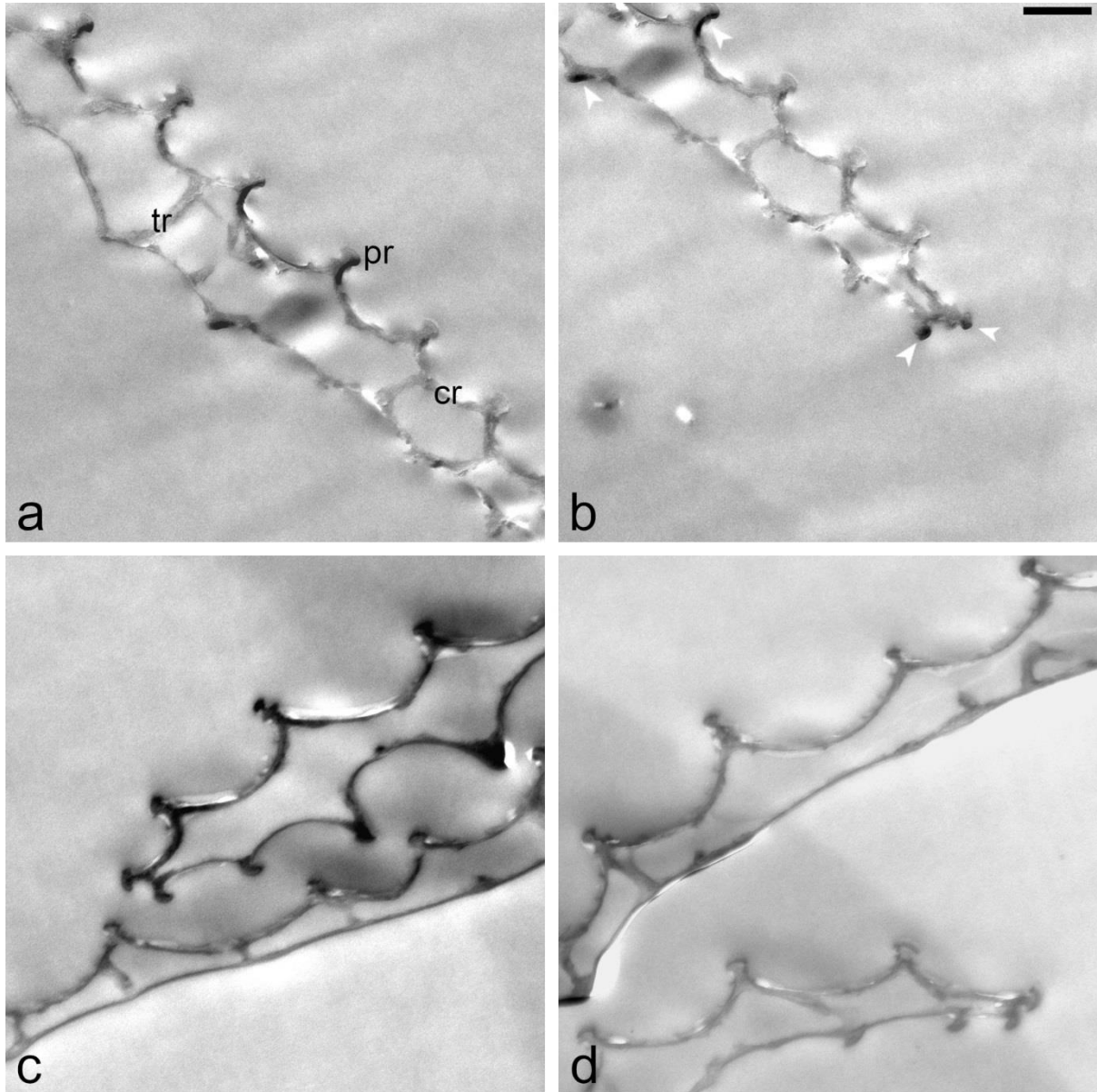
Figure 17: TEM images of scales from a male moth

(a) Scale of male moth of *Kessleria burmanni* from the Blaser, showing regularly structured upper lamina (abwing side) and irregularly structured lower lamina (adwing side). Heterogeneous pigmentation can be seen; (b) edge of scale (a) with pigmentation in some primary ridges (arrowheads) on the upper and irregularly structured lower lamina (adwing side). (c) two heterogeneously pigmented scales of male *Kessleria burmanni* from the Blaser.

Only edge is shown from upper scale. It exhibits no trabeculae, higher pigmentation and more structured lower lamina (adwing side) than the center of the second scale; (d) the two scales of (c) from another section. The center of the upper scale and the edge of the second/lower scale are now shown. The upper scale exhibits in its center a nearly plain lower lamina (adwing side) and a lower pigmentation level, whereas the edge of the lower scale shows more of an irregularly structured lower lamina (adwing side) and no pigmentation at all. Brightness and contrast adjustments were made. Scale bar for all images: 250 nm. Abbr.: *tr* trabecula; *pr* primary ridges and *cr* crossribs.

Spectrometry

All measurements of two samples of forewings from both sexes of *Kessleria burmanni* reveal extremely high remission in the ultraviolet spectrum (**Figure 18 and 19**). As the maxima in both, male and female species, are closely spaced, they must be caused by structural properties of the scales. The maxima within the visible range are just around 10 (%) to 15 % - diffraction grating is brought about by spectrometric analysis on the dorsal/abwing as well as the ventral/adwing side of the wings of both sexes. In males and females and on either side of the wing an increase from the visible range to the infrared spectrum can be observed. The dorsal/abwing side of the wing shows higher remission rates (approximately 30 % to 40 %) than the ventral/adwing side (**Figure 19**). Furthermore, female moths exhibit higher remission than their male counterparts (**Figure 18**). In female specimens remission is low at the base of the wing, extremely high in the center and decreases again at the fringe (**Figure 18 a**). The same pattern holds true for the male moths; however, we were not able to measure the base of the wing in one sample, because it still stuck to the animal (**Figure 18 b**) or was too crooked to make a precise statement. The decreased remission at the wing base and at the wing end may be also due to technical problems, because our specimens were so small, that the sensor just covered half of the sample site. Measurements of the household aluminum foil were unusable.
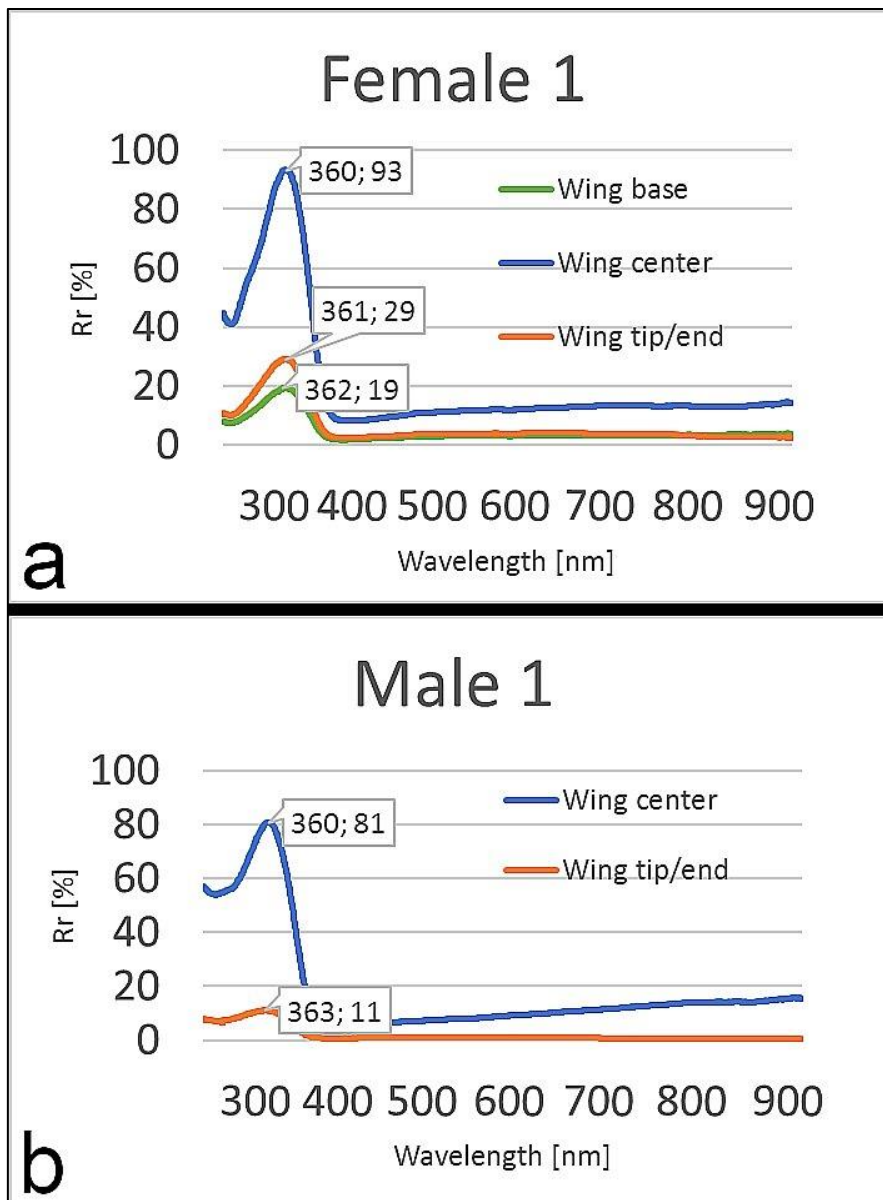
Figure 18: Spectrometric data of a female and a male specimen of Kessleria burmanni scales

(a) Spectrometric data of three different measurement positions: base, center and end of forewing of female moth of *Kessleria burmanni*. On the x-axis is shown the wavelength (nm), on the y-axis the relative reflection (Rr) in %. The base of the forewing has its peak at 362 nm with a reflection of 19 %, the highest peak of the whole wing can be measured at the center (360 nm, 93 % reflection). Reflection is again declining at the wing end. There the peak is around 29 % at 361 nm. A slight shift into the infrared spectrum can be seen; (b) spectrometric data of two different measurement positions (wing center and wing tip/end) of the forewing of a male *Kessleria burmanni*, showing the wavelength in nm on the x-axis and the relative reflection (Rr) in percentage on the y-axis. The longest wavelength measured at the wing center is 360 nm (81 %), on the wing end 363 nm (11 %). A shift towards the infrared is visible in the wing center. Both specimens were sampled at the Blaser.
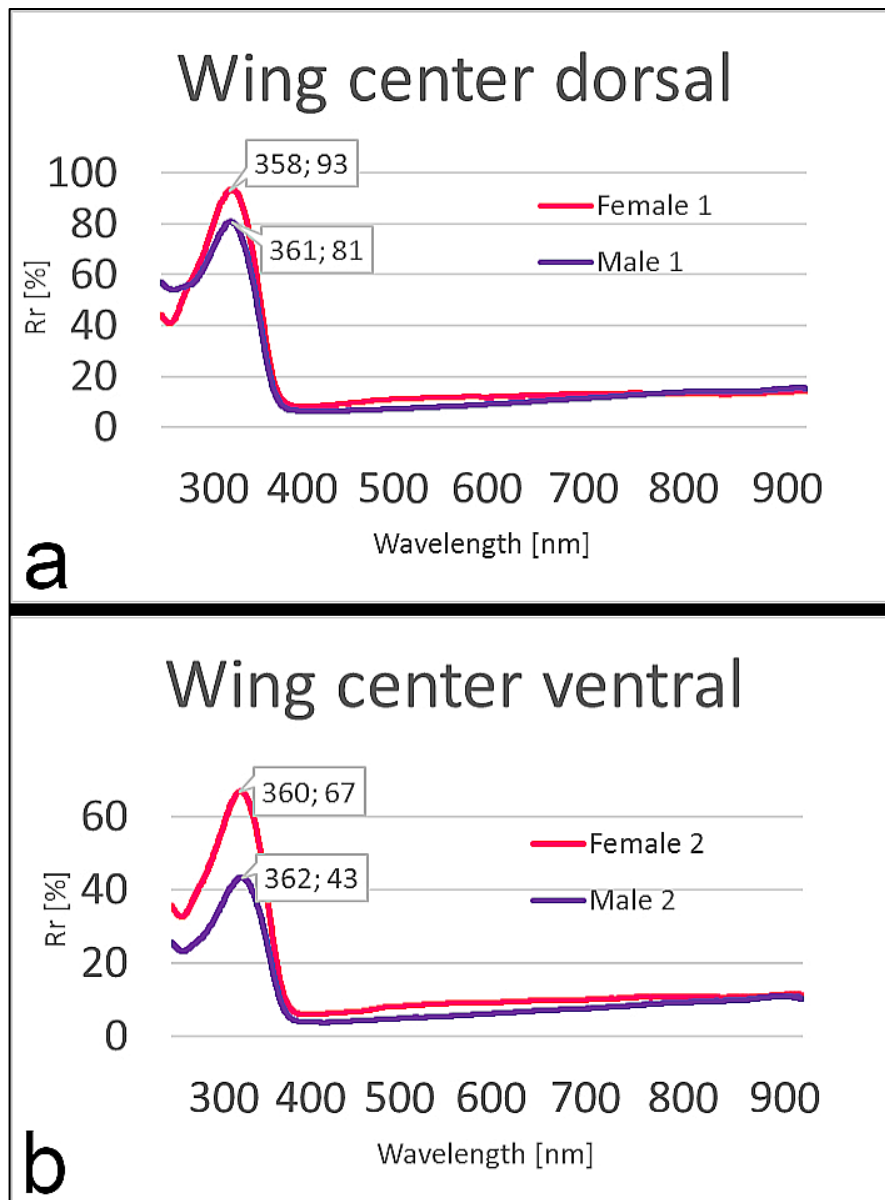
Figure 19: Spectrometric data of the dorsal/abwing side and the ventral/adwing side of a female and a male moth

(a) Comparison of measurements at the wing center on the dorsal side of a male and a female specimen, showing the wavelength in nm on the x-axis and the relative reflection (Rr) in percentage on the y-axis. Both samples exhibit a peak in the ultraviolet spectrum, the female at 358 nm (93 % reflection), the male at 361 nm (81 %). In both specimens a shift into the infrared can be noticed; (b) comparison of measurements at the wing center on the ventral side of a male and female specimen, showing the wavelength in nm on the x-axis and the relative reflection (Rr) in percentage on the y-axis. The female sample reaches its peak at 360 nm (67 % reflection), the male at 363 nm (11 %). Both samples exhibit increasing reflection of about 15 % towards the infrared range. Both specimens were sampled at the Blaser.

Flight analysis of *Kessleria burmanni*

Flight-Patterns of the females

In comparison to the males, the female moths of *Kessleria burmanni* give the impression that they are struggling with flight, a conclusion fostered by their body wing ratio. Therefore, for a long time scientists have assumed that they – apart from walking – only use a jumping sort of locomotion, without any support from the wings (Randl, 1993). In a strict sense, this claim cannot be upheld any longer as slow-motion video tracking testifies to the contrary: The females of *K. burmanni* do use their wings while jumping, but to which extend they contribute to motion still needs to be discussed (**Figure 21**). Generally depending on the length of the jump, the females show an average wing beat frequency of about 35 Hz at rt.
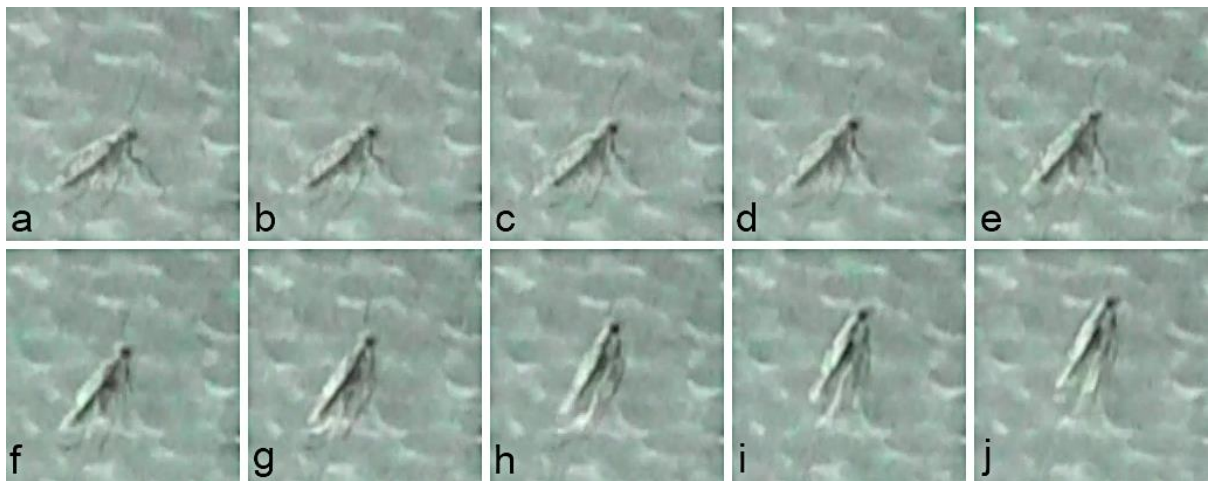
Figure 20: Jumping start of the flight of a female moth

(a-e) Preparations for jump of female moth of *Kessleria burmanni* (0 dph) form the Blaser, building up tension as seen from the side; (f) shortly before take-off; (g-j) actual jump of female moth with video footage of body tension during jump/flight. The female moth is meanly 0,5 to 0,6 cm long. Brightness and contrast adjustments were made. The original video contains evidence of the wing beats of the moth during jump/flight. Link: https://goo.gl/XvFCyS

Figure 21: One wing beat of a female moth

(a–y) Nearly complete wing beat of female *K. burmanni* (0 dph), sampled at the Blaser. The animal rotates from side to front p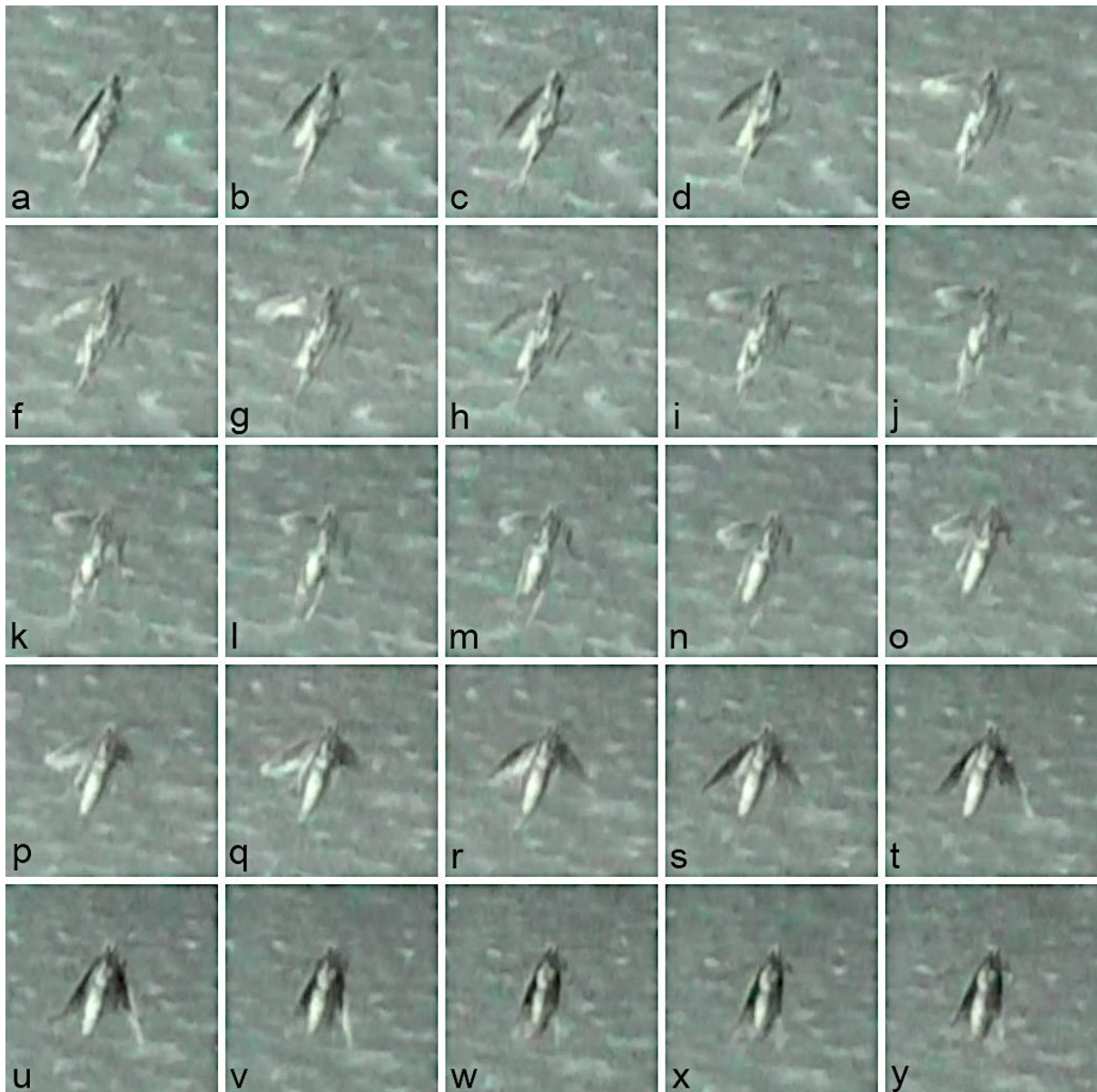osition during flight. (a and b) starting jump with high body tension and completely outstretched legs; (c) beginning of wing beat; (d-k) wings turning dorsally up with animal starting to turn around (e); (l) upstroke of wings and complete rotation to front position; (m-y) wings turning ventrally down; (x) complete downstroke of wings; (y) wings starting to turn up again. All images have been aligned so that the animal is in the center. The female moth is meanly 0,5 to 0,6 cm long. Brightness and contrast adjustments were made. In the original three second video more wing beats of this moth can be seen. Link: https://goo.gl/XvFCyS

Flight-Patterns of the males

Basically, the males showed a vastly better flight than the females. They reach a wing beat frequency of about 43 Hz on average at rt. As mentioned above, some of them flew until they

reached the top glass of the wind tunnel, where in the experiments our illumination source was placed. However, most of them don't seem very much inclined to fly on any given occasion. For the most part, their very short flights can best be described as helical.

Figure 22: Flight of a male moth as seen from the front

(a–y) One full wing beat of a male *K. burmanni* (0 dph) from the Blaser, during forward flight as seen from the front. Starting from downstroke position, (a) to fully outstretched wings h), from up-stroke, dorsally folded (m) and fully outstretched (r) to newly closed wings (y). (d-o) See the amount of reflection of light due to scales of animals. All images have been aligned so that the head of the animal is in the center. The male moths´ length is below 1 cm. Brightness and contrast adjustments were made. The original three sec. video contains the upward flight of the moth towards the light source. Link: https://goo.gl/zsfzcg
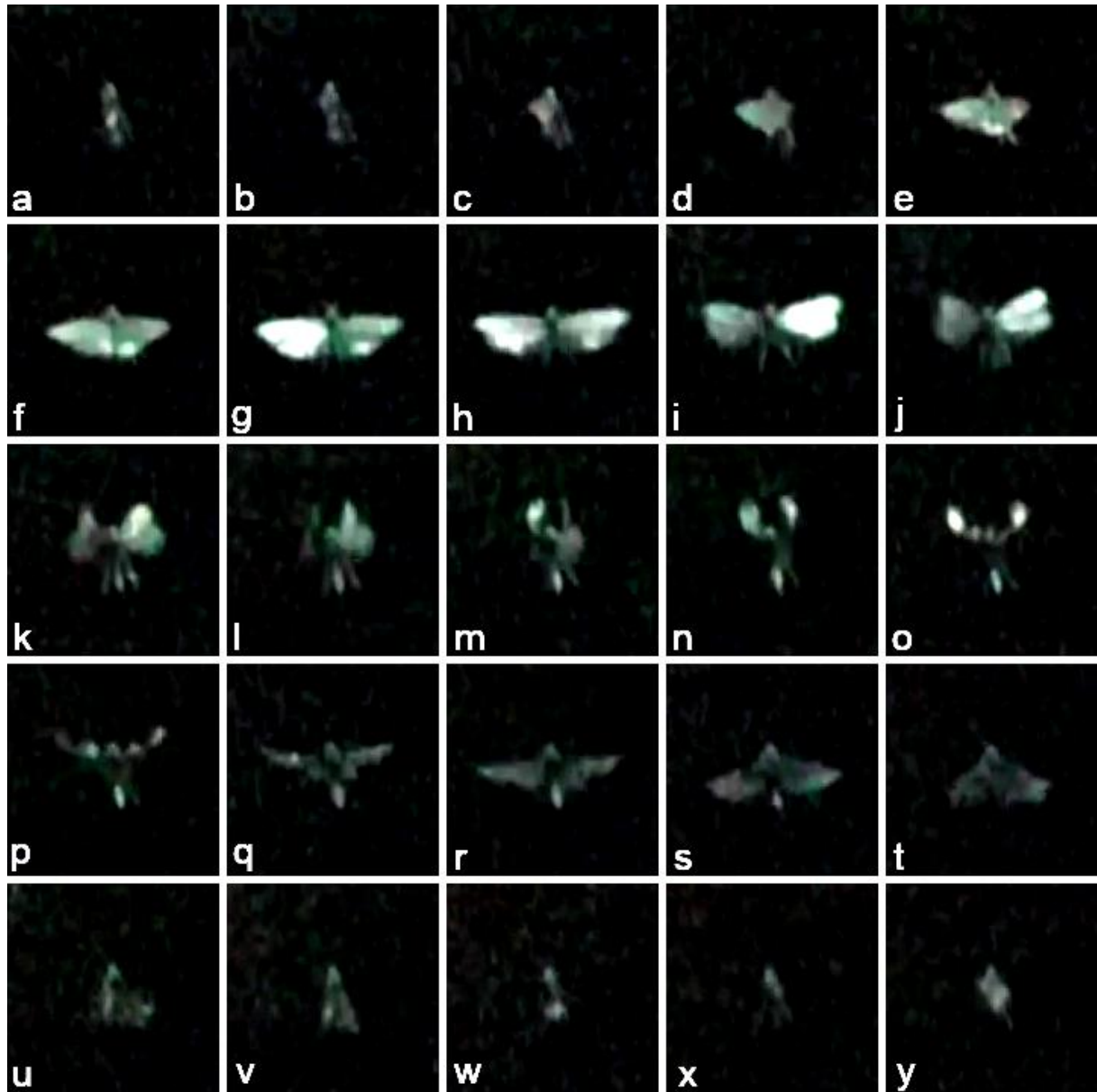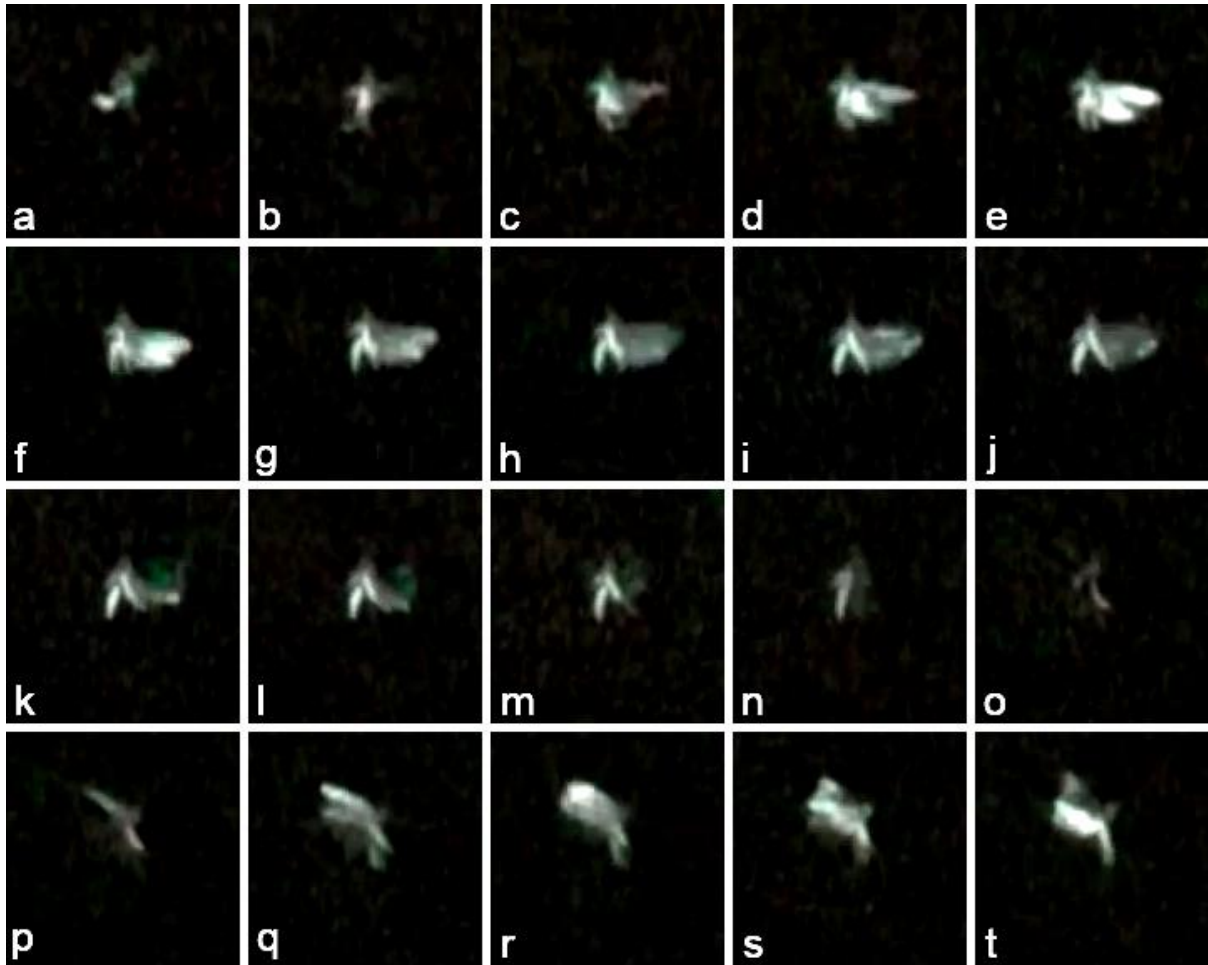
Figure 23: Flight of a male moth as seen from the side

(a–t) One full wing beat of male *K. burmanni* (0 dph), sampled at the Blaser, in forward flight towards light source as seen from the side. Starting with downstroke (a), continuing with upstroke (j) finishing with downstroke (t). The animal appears brighter when reflecting the light. Images taken from the same video as figure 22. All images have been aligned so that the head of the animal is in the center. The male moths´ length is below 1 cm. Brightness and contrast adjustments were made. Link: https://goo.gl/zsfzcg

Discussion

Classification of *Kessleria burmanni*

A total of seven *Kessleria* species is known to inhabit the Austrian Alps (Huemer and Tarmann, 1992). *Kessleria saxifragae* (Stanton, 1868) is described with a forewing length of 7,2 - 8,6 mm in male and 6,5 - 7,4 mm in female moths. The exuvia of the pupa is light brown, the larvae between 10 - 12 mm long, light green with a light red line at the dorsal side. The forewings of *Kessleria caflischiella* (Frey, 1880) are nearly of the same size as those of *Kessleria saxifragae,* ranging from 6,9 - 8,3 mm and 6,1 - 7,1 mm. Likewise, the exuvia is light brown, the larvae – between 11 - 12 mm in longitude - are of light grey-green appearance and also show a light red line on the dorsal side. In *Kessleria petrobiella* (Zeller, 1868) the male and female moths exhibit nearly the same forewing lengths (6,3 - 8,0 mm in males vs. 6,4 - 7,8 mm in females). Once more, the exuvia is light brown, and the yellow-green larvae with a red-brown dorsal line range from 11 – 12 mm in extension. Endemic in the northeast Alps is *Kessleria hauderi* (Huemer and Tarmann, 1992). The forewings of the male moths amount to 8,0 - 9,3 mm, whereas the wings of the females are 6,1 - 7,1 mm long. The exuvia is also light brown. The larvae are 10 - 12 mm in length and look dirty dark green with a red-brown line on the dorsal side. The males of the species described by Burmann (1980), *Kessleria nivescens*, have a forewing length of about 8,0-9,6 mm, whereas the forewings of the females are a little bit smaller (6,9-8,0 mm). The exuvia of the pupa is black-brown. Their larvae appear dark and dirty in green-brown colors and are somewhat between 12 -13 mm long. In *Kessleria alpicella* (Staınıon, 1851) the male moths exhibit a forewing length of about 7,0 - 8,95 mm and the females about 7,3 - 7,6 mm. The exuvia is light yellow and brown. The green larvae are 12 mm long (Huemer and Tarmann, 1992).

In *Kessleria burmanni* (Huemer and Tarmann, 1992) the forewings of the females on average are clearly shorter (0,503 cm) than those of the male moths (0,727 cm) (**Table 7 and Figure 10**). Differences in front and back wing lengths of specimens living in different habitats do, however, occur (**Table 8**). The exuvia is light brown, the larvae about 10 - 11 mm long, of a dark green-brown color with a lighter ventral side and a broad and dark red-brown dorsal line (**Figure 5 b and Figure 6 c, d and e**).

In accordance with Dr. Peter Huemer, the classification of our specimens has been done with regard to wing length – including the brachypterious female moths –, coloring in both, larvae and adults, sampling site (distribution) and habitat, as well as host plants of larvae. Vice versa

this scheme can also be used as a test for any new-found specimen. After checking all the parameters, we were able to conclude that any given sample belongs/doesn´t belong to *Kessleria burmanni*.

Cultivating conditions

Starting from some 26 larvae, we managed to raise the eight adults mentioned above (**Table 3 and Table 4**). This might be due to the cultivating conditions in our laboratory on the one hand and the sampling of early larval stages on the other hand. As the study of Randl (1993) suggests, it would have been better to separately keep the larvae in boxes or petri dishes to guarantee exact control over quantities at any time. Additionally, cultivation would have been preferable in a climate cabinet with regulated temperature – we kept the animals at rt and under long-day conditions. Furthermore, lining petri dishes with agar is worthwhile to prevent plants and larvae from drying out, but it can also bolster the growing of mold. Because some of our plants drying out (**Figure 6 a**) and the consecutive reduction of food supply, we had to transfer our larvae to fresh plants. In this process maybe some early stage larvae escaped our notice or got killed or injured in the transfer.

Decreased larval findings

Another critical issue is the reduced occurrence of *K. burmanni* in general. Compared to the data reported in Randl (1993), our work brings to the foreground an obvious decrease in larval findings, sparse populations of animals at the mentioned sampling sites or even the loss of previous sites, e. g. at the Nordkette **(Figure1 c)**. This condition may have been caused by anthropogenic influences, such as grazing or manuring the soil, changed weather conditions over the last couple of years or effects of climate change. Furthermore, a heavy decline in abundances and diversities of insects can be observed these days on a global scale (Hallmann et al., 2017).

Extremes and increasing average temperatures – both connected to climate change –can vastly influence the individual performance and fitness of animals as well as population dynamics. It effects the distribution and abundance of species as well as the structure and function of ecosystems as a whole (Buckley and Kingsolver, 2012; Parmesan et al., 2000). To examine the possible impact of exceptional weather conditions, weather data – above all temperatures and wind velocities – were gathered from three different services and their

stations on the Patscherkofel, the Hafelekar, the Sattelberg and in Steinach am Brenner (see Material and Methods).

For the last two years no evidence whatsoever of outstanding weather events could be found. Strikingly, however, in 2016 the average temperature on the Hafelekar was measured to be approximately five degrees higher than that at the other stations at the same time. One is tempted to speculate about the possible negative effect higher average temperatures can have on the condition and fitness of *K. burmanni* in general. Warm temperatures in combination with windy conditions do in fact promote dehydration of animals and their feeding plants. On the other hand, at the said stations the wind was strongest in the spring and autumn months and varied from low during twilight hours to higher throughout the day. For the time being and in absence of a more probable and thorough investigation into the reasons climate-based factors as well as anthropogeny might serve as a tentative explanation for the visible decline of larvae findings at the Nordkette as compared to Randl (1993).

Lifespan

One of the topics most studied in human welfare is longevity. Lifespan differs between various species and between insects and larger animals in general (Holm et al., 2016). In the realm of animals scientists have primarily been concerned with a limited number of model organisms such as the nematode *C. elegans*, *Drosophila melanogaster* (the fruit fly) or *Mus musculus* (the house mouse) in recent years (Austad, 2010; Jones et al., 2014; Ricklefs, 2008). Insects are of special interest in this respect as they provide possibilities for investigations into longevity in high quantities and various qualities (species) (Holm et al., 2016).

Our specimens, kept in the fridge at around 6 °C to simulate Alpine temperatures, showed an average lifespan of 28,7 days. The highest lifespan could to be found in a female moth (8w1d), whereas male moths lived considerably shorter with a maximum lifespan of 3w5d. The shortest period of life in both, male and female moths, was measured to be 1w6d (**Table 4**). By comparison, the study of Holm et al. (2016), covering some 2472 individuals from 98 geometrid species, mentions an average post-capture lifespan of a moth under laboratory conditions to be seven days. In their experiments they kept the moths in two opposite environments: cool and warm conditions. Females living under cool conditions on the average lived longer (9d) than those held in warm surroundings (5d). The same is reported to be the case for male moths living in warm (8d) and cool (4d) environments.

On the one hand, evolutionary models of herbivore insects suggest the duration of life in female adults to be negatively associated with larval diet breadth. This controversial view presupposes imago-insects exerting some sort of adaptive plant selection as response to larval performance. Some biologists, however, take the contrary view by arguing that larval diet breadth is in itself determined by evolutionary changes in the lifespan of the female insects of a species (Jervis et al., 2007). *K. burmanni* displays adaptive host plant selection to *Saxifraga caesia* and *Saxifraga oppositifolia*, but most of our female moths lived longer than the male ones.

Patterns of Flight
Fundamentals

For a conception of flight it is necessary to realize that the wings of flyers support their weight against the force of gravity and therefore the upward lift must compensate the weight of the flyer. The carrying capacity of wings is determined by size, angle, air-density and air speed (Tennekes, 2009). This is to say that not only the structural dynamics of the wings, but also aerodynamic features decisively influence the flight performances of insects (Nakata and Liu, 2011). Additionally, body temperature plays a decisive role (Clench, 1966). Mechanosensory halters, e.g antennae, allows the insect to make use of Coriolis forces and thus help stabilize the flight in the course of maneuvering (Sane et al., 2007). They also support unexpected behavior of insects, such as turning or rolling over (**Figure 8 and 9**).

The Reynolds number (*Re*) features prominently in the scientific description of the flight behavior of any object. Taken from fluid mechanics it generally describes how viscous forces act on a body and denotes the dimensionless ratio between inertial and viscous forces. Low *Re* therefore is characterized by a nearly laminar flow of the fluid almost without turbulences, a high Reynolds number represents the opposite (Purcell, 1977). A function of *Re* is the aerodynamic attainment of an airfoil. As it varies with body size, smaller objects should face larger viscous forces within the fluid, but because insects have more than a few milligrams of body mass, the effect of body size on flight behavior should be minimal (Lehmann, 1999). Small insects in general face an intermediate *Re* regime of about 10 < *Re* < 1000 during flight (Dickinson and Götz, 1993).

Finally, weather conditions influence flight performance of insects, e.g. wind dominates the lives of insects, because they might easily be distracted during flight. Therefore, the very

smallest of them in order to fly have to wait for the twilight hours with subduing winds or stick close to the ground during flight (Parry, 1951; Tennekes, 2009). *K. burmanni* seems to prefer flying in the morning hours immediately after sunrise (Huemer and Tarmann, 1992).

Brachyptery

The females of *Kessleria burmanni* are brachypterous, which means that they have reduced wings (Huemer and Tarmann, 1992). Brachyptery in Lepidoptera is known to be widespread in windy habitats, on islands, e.g. Hawaii, Maui Island and New Zealand, and in areas with poor vegetation. There the animals use a form of "jumping" instead of flying (Medeiros, 2008; Patrick and Green, 1991). Huemer and Tarmann (1992) and Randl (1993) argued that for jumping the brachypterous females of *K. burmanni* did not use their wings at all. Slow-motion tracking in our self-made wind-tunnel, however, brought about new results and insights as it showed that also the female moths do actually use their wings while "jumping" with an average wing beat frequency of 35 Hz.

Wing beat frequency

When interpreting the slow-motion tracks of the male moths a similarity to the flight of hummingbirds or even bats came to our mind. According to studies of Bullen and McKenzie (2002) with 23 species of Australian bats, these animals own a wing beat frequency between 4 and 13 Hz – hummingbirds can even reach average frequencies between 50 and 60 Hz (Clark Christopher James, 2009; Greenewalt, 1960). Contrastingly, our specimens displayed wing beat frequencies of about 43 Hz in male and some average 35 Hz in female moths. All measurements were done at rt. and during forward flight inside the wind tunnel of our own construction. (**Figure 21, 22 and 23**).

In fauna the light weighted mosquito stands out with the highest wing beat frequency of some 300 Hz to 500 Hz on average (Gibson and Russell, 2006). Another light weighted flyer in the class of insects is *Drosophila sp.* With a wing beat frequency of meanly 190 to 212 Hz in forward flight and about 209 Hz for hovering (Lehmann and Dickinson, 1998). Similarly, honeybees show frequencies of about 230 Hz during hover-flights (Altshuler et al., 2005) and 136 Hz - 202 Hz in forward flight depending on thorax temperatures (Esch, 1976).

The world of moths covers a vast spectrum of wing beat frequencies as size and body weight differs from species to species. Individuals from the *Saturniidae*, the family containing the largest species of moths, have heavy body masses and reach average wing beat frequencies

of about 23 Hz. In contrast, specimens from the *Sphingidae* family, also known as hawk or sphinx moths, show wing beat frequencies of 41 Hz on average, although endowed with considerable body mass (Bartholomew and Casey, 1978). Our specimens, then, weighed some 1,9 mg on average with a standard deviation of 0,4 mg (**Table 3 and 5**) – still, their wing beat frequencies were quite high proportionally even if they need to be considered low if compared to mosquitos, which are generally of equal or lesser weight depending on the species. Butterflies, as a last example, own lower wing beat frequencies than moths as their wings are considerably larger and thus wind loading is low (Heinrich, 1993).

As can be gathered from the previous statements and data wing beat frequency is indirectly proportional to the size of the animal. In their study on 65 bat species Norberg and Norberg (2012) found ample proof for the correspondence of decreasing frequencies and increasing body mass. They also mention that the body mass dependency among birds is like that of the bats, whereby, the wing beat frequency is always higher in birds. The same correlation also holds true for different bee species (Unwin and Corbet, 1984). This leads to the following conclusion: If an insect is small in bodily dimensions, the wing to body mass ratio and the Reynolds number will be small as well, but the wing beat frequency will increase (Shyy et al., 2016).

Function of scales and bristles

An insect's life and biology are mostly determined by its exoskeleton, which adopts functions of the body walls, limbs and the respiratory system. Surfaces of exoskeletons can be lined with e.g. scales, bristles and extremely fine hair (**Figure 11, 12 and 13**). Scales are not said to be universal among insects, whereas bristles can be found in all arthropods. Both, bristles and scales (**Figure 14, 15, 16 and 17**), are called macrochaetes, the small pieces of hair are called microchaetes (Ghiradella, 1998). Scales and bristles with their micro – and nanostructures (**Table 10, Figure 15, 16 and 17**) fulfill several functions (Bálint et al., 2007; Ghiradella, 1998), e.g. filtering food (Fry and McIver, 1990).

In sexual communication, scales and bristles are amid other things responsible for the production and distribution of various insect pheromones and scents, as well as for producing ultrasonic sounds on an extremely low intensity level (Egelhaaf et al., 1992; Ghiradella, 1998; Nakano et al., 2008; Vane-Wright, 1972; Wasserthal and Wasserthal, 1977). They also effect sexual communication by setting up premating barriers between different species or

populations for approaches that go beyond the purely physical or quasiphysical level (Bálint et al., 2007; Isaacson, 2017). Signaling of that sort does not only influence interactions of the same sex (male – male), but also hetero (male-female) communication. For example, during courtship, male specimens produce pheromones to "activate" the females for copulation. Those pheromones stick to piliform scales attached to the males´ "fur" as to keep the scent attached to the wings as long as possible (Bálint et al., 2007). In *Orgyia leucostigma*, the white – marked tussock moth, a pheromone extract from female body scales triggers off male copulatory behavior (Tilley et al., 2002).

In sexual communication among insects also wing color plays an important role (Kemp, 2008; Wiernasz, 1989). The micro- and nanostructures of some of the scales are responsible for the shiniest and most animating colors in nature. Only during interaction with light are they generated by a combination of multilayer interference, photonic crystals, optical gratings and other optical structure (Kolle et al., 2010; McNamara et al., 2011). Not only is signaling of that kind important in male – male interaction (e.g. to recognize rivals), but it is also decisive in male – female interaction, where e.g. higher remission rates of either male or female moths instigate detection of the other sex (**Figure 18 and 19**) (Kaaber et al., 2009; Kemp, 2008).

In addition to that, scales and bristles are also home to various sense organs or sometimes hold and display exudates as a means of cooperation with other insects, like e.g. ants (Kitching and Luke, 1985). Scales and bristles also serve the purposes of warning, recognition and camouflage – all aspects greatly influenced by wing-color (Ghiradella, 1998; McNamara et al., 2011). Protection against predators is fundamental to scales and bristles, e.g. against spider webs, producing wax-like "shields" of a tasteless or even toxic nature, or by absorbing sound and thus being indistinguishable for e.g. bats (Ghiradella, 1998; Zeng et al., 2011).

As far as thermoregulation is concerned, they protect the insect against heat loss or overheating by either reflecting the light or absorbing solar radiation with the help of pigments like melanin (Ellers and Boggs, 2004; Ghiradella, 1998; Zeng et al., 2011). Furthermore, scale structures work as solar panels, whereby the substance of melanin may be adapted to the animal´s habitat (Biró et al., 2003; Ellers and Boggs, 2004). It is known that melanin granulates in the brown dorsal wings of Lycaenid butterflies directly facing the sunlight, hence absorbing more energy than the wings of blue colored species (Biró et al., 2003). Flyers with a higher level of melanin are believed to be more active under cool or cloudy weather conditions than

lighter individuals and thus better prepared to face their predators (Roland, 2006) This may well be the reason for increased melanization in animals living in elevated or high surroundings (Guppy, 1986; Karl et al., 2009). On the other hand, lighter flyers may have an advantage in sun exposed habitats, where overheating is much more of a danger (Ellers and Boggs, 2004; Gibert et al., 1998).

Melanin also plays an important role for sexuality (Wiernasz, 1989) and in defense of parasites (Siva–Jothy, 2000). The females of most temperate species are of brownish color at the dorsal side to obtain more energy and keep the perfect body temperature for the production of as many fertile eggs as possible (Bálint et al., 2005). Conversely, differences in wing melanization are known to be the cause for the males showing greater proneness to fly (Karl et al., 2009). Finally, melanin also protects against UV-radiation by absorbing the light (Giraldo et al., 2008; Kertész et al., 2006). According to Huemer and Tarmann (1992), specimens of *K. burmanni* contain different amounts of melanin as a response to life in different habitats (**Figure 10**) Additionally, sexual dimorphism can be observed between the melanization of male and female moths, the male specimens generally appearing darker than the female ones (**Figure 10, 11 and 12**).

In flight scales and bristles smooth the airflow and, thus, improve aerodynamic efficiency (Ghiradella, 1998; Slegers et al., 2017). It is known that scales exhibit hydrophobic features which more or less waterproof the insect and, therefore, allow the animal to fly even during light rain (Yoshida, 2014). Scales and bristles often increase the wing area by simply extending the wings. Furthermore, they help to link the front and the back wings and increase lift and thrust during flight (Ghiradella, 1998). According to Bernoulli, the air over the top surface flows faster than the air below. As a result, the pressure on the bottom surface has to be higher than on top. Conversely, a wing produces a downward deflection in the surrounding air by a kind of suction on the top surface of the wing. This leads to a downward deviation of the air passing the wing, and a partial vacuum on top lifting the flyer up. The differences in pressure can be computed with the Bernoulli formula (Tennekes, 2009).

In *Kessleria burmanni* can be observed a lower lamina (adwing side) of the scales in parts structured irregularly (**Figure 16 and 17**). According to Ghiradella (1998) scales are ornamented on the upper/abwing side and featureless on the lower/adwing side. However,

more recent studies have shown that lower laminas, especially in moths, can also exhibit structural colors and therefore cannot be featureless (Stavenga et al., 2014, 2018).

Adaptations to the Alpine Climate

According to Sømme (1989) insects need considerable adaptations to live in the harsh Alpine climate. In *Kessleria burmanni* various morphological adaptations to their Alpine environment can be observed.

Firstly, their reduced body size is of great advantage for finding shelter in microhabitats, especially in their host plants *Saxifraga caesia* and *Saxifraga oppositifolia*. The reduction in body size may be due to longer development times and shorter growing seasons in the Alpine environment (Frazier, 2007). Reduced body size influences the development time (Gillooly et al., 2002), the metabolic rate (Gillooly et al., 2001), the population growth rate (Savage et al., 2004) and the flight energetics (Dudley, 2002) of insects.

Secondly, in female moths sexual dimorphism relating to wing atrophy and brachyptery is widespread. Female specimens are endowed with shorter wings than the male ones and thus only show distorted flight or "jumping" motion. This leads to the presumption that the females of *K. burmanni* save part of their energy to produce as many vital eggs as possible. In this respect then the female moths are more valuable and more in need of protection, e.g. by not letting them get blown away by wind during flight.

Thirdly, the coloration pattern of *Kessleria burmanni* is of great advantage in an Alpine environment. With their white and brownish patterned wings they are well adapted to their habitat and perfectly protected against predators. Their full-body scaling serves against heat loss, whereas the white part of the wings may be of advantage against overheating.

Additionally, higher melanization in males brings about higher flight activity (Karl et al., 2009) – another important factor for procreation, because the females are somewhat less agile and refrain from actively encountering the males. Apart from that, Melanin also protects against UV-radiation.

Kessleria burmanni is also known to have many behavioral adaptations to their habitat, e.g. seeking shelter in warmer microhabitats or under rocks to regulate body temperature. It is also known that the insects are most active in the early morning hours when there is nearly

no wind (Huemer and Tarmann, 1992). This might protect them against predators or help them maintain their water-balance. When exposed to strong winds, they remain in some sort of "parking position", aligning themselves in the direction of the wind, "freezing" their movement with antennae and legs clinging to the body, the latter always kept in a tilted position. This behavior protects them from getting blown away by strong winds. Additionally, the moths seem to favor vertical sides, which might be due to their habitat, including hard rock and their host plants. Finally, *Kessleria burmanni* has a multi-annual life-cycle with two overwintering stages (Randl, 1993).

Conclusion and Outlook

Kessleria burmanni has escaped scientific notice for quite some time. Maybe its distribution, its prevalence in remote Alpine regions and its small dimensions have contributed to a lack of scientific interest in its patterns of behavior, above all flight. It needed an arrangement of tools especially set up, wind tunnel experiments linked to slow-motion tracking, to finally prove that the female moths of this species do beat wings while "jumping".

But can this properly be called "flight"? What constitutes flight? What is the difference between a jump, where wings do beat, and flight? Flight as a form of movement allows the flyer to change its location without any contact with the surface by using wings. The direction of the movement in *K. burmanni* females seems to be destined by the final positioning of the legs right before the jump occurs, thus their movements seem partly somewhat uncontrollable. Although exhibiting wing beat frequencies whilst jumping, they remained quite low (35 Hz) considering the body size and the weight of the moths. Furthermore, the wing beats displayed no impact, e.g. on the direction or stability, of the "flight". To clarify the "flight/jump" issue further studies may be of advantage.

Although, we were able to raise eight moths and keep them alive up to eight weeks and one day in the laboratory, a fair bit of work is still needed to improve the cultivation techniques of *Kessleria burmanni*. One out of eight moths died from dehydration before experiments had even started. Furthermore, a lot had to be learned about feeding and nutrition of the adult moths as we made our way from trial – and – error at the beginning. Eventually, feeding droplets of grape juice came out as a suitable way of sustaining the moths. We did not, however, succeed in actually breeding our specimens – there more work needs to be done to study behavioral patterns of *Kessleria burmanni* on a larger scale.

A few questions still remain unsolved by this work. It was for the first time that during the course of the experiments an irregularly structured lower lamina of a scale came to our notice. As the structures of the upper lamina of scales play an important role in many respects it seems reasonable to think that the same is also true for the lower lamina. But what is its function really? What does it contribute to any of the modes of adaptation discussed in this work so far? Or does it have some completely different purpose unthought-of of before?

In addition to that, it is already known that the flight behavior of the insects differs with age (Levenbook and Williams, 1956). Our experiments also slightly pointed towards a change of activity corresponding with age, but it remains to be thoroughly studied at which age they really show their most active flight behavior. Here also further quantitative analysis may be desirable.

As discussed above, *Kessleria burmanni* morphologically is well adapted to the Alpine environment. It would be of great interest, too, to find out if the insect shows signs of adaptation in a physiological sense. Do the moths also respond internally to external stimuli prevalent in their predominant habitat?

Mention has been made of the fact that the forewings of *K. burmanni* reveal extremely high remission in the ultraviolet spectrum and a specific remission pattern over the whole wing. Is remission used for inter and/or intraspecific communication, perhaps even for sexual communication?

To prove the relevance and the reproducibility of some of the results it would be necessary to carry out the same experiments again several times. For the SEM scales only one female moth was studied - it would definitely be interesting if the male specimens also show the same or similar irregular structures on the lower lamina. For more exact results of the spectrometric data, it would in general be reasonable to repeat the measurements with better suited, e.g. smoother, forewings.

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Abbreviations

Abbr.:	Abbreviation
cm:	Centimeter
CO_2:	Carbon dioxide
d:	Day/Days
dph:	Days post hatching
e.g.:	For example
Hz:	Hertz
K. burmanni:	*Kessleria burmanni*
Km/h:	Kilometers per hour
m:	Meter
mg:	Milligram
mm:	Millimeter
µm:	Micrometer
nm:	Nanometer
O_2:	Oxygen
rt:	Room temperature
sec:	Seconds
SEM:	Scanning electron microscopy
TEM:	Transmission electron microscopy
UV:	Ultraviolet
w:	Week

Keywords

Kessleria burmanni, Moths, Lepidoptera, Biometric Measurements, Wind Tunnel, Flight, Flight Dynamics, Locomotion, Low Reynolds Number, Wing Beat Frequency, Wing Atrophy, Brachyptery, Feeding, Scale Function, Adaptations, Alpine Climate, Sexual Dimorphism, SEM, TEM, Spectrometry.

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