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***Stenomicra* sp. flies (Diptera: Periscelididae) on rolled leaves of
Heliconia sp. and *Calathea* sp.**

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ABSTRACT

Stenomicra are a genus of small, often yellowish flies of the family Periscelididae (Diptera). The *Stenomicra* I studied live in leaves of *Heliconia* sp. and *Calathea* sp. in Monteverde, Costa Rica. While not much is known about their biology, I suspected that some factors may influence whether or not they will be found on a particular leaf. I proposed that the amount of matter found within the rolled leaves, other arthropods within the leaves, and the stage of the rolled leaf affect whether or not the *Stenomicra* flies inhabit them. I found that none of the above factors contribute to whether or not you will find them on a rolled leaf; as long as it is even a little rolled, it is likely that they will be there, and they do not require a certain amount of matter in order to inhabit a leaf. I found that number of arthropods in a leaf does not affect the amount of matter in a leaf as well. I also suspected that the ephemeral phytotelmata of these rolled leaves may serve as the larval home of *Stenomicra*, so I clipped off the bases of the leaves so that I could search for larvae under a microscope. I found two fly larvae in the matter inside the rolled leaves, one of which is *Stenomicra*. Because of this, it may be possible that the *Stenomicra* that inhabit these rolled leaves complete their entire life cycle within them.

**Moscas *Stenomicra* sp. (Diptera: Periscelididae) en hojas enrolladas de
Heliconia sp. y *Calathea* sp.**

RESUMEN

Stenomicra es un género de moscas pequeñas, a menudo amarillentas, de la familia Periscelididae (Diptera). Los individuos de *Stenomicra* que estudié viven en hojas de *Heliconia* sp. y *Calathea* sp. en Monteverde, Costa Rica. Si bien no se sabe mucho sobre su biología, sospeché que algunos factores podrían influir en si se encontrarán o no en una hoja en particular en estas plantas. Propuse que la cantidad de materia almacenada encontrada dentro de las hojas enrolladas, la presencia de otros artrópodos dentro de las hojas, y la etapa de la hoja enrollada afectan si las moscas *Stenomicra* habitan esas hojas o no. Descubrí que ninguno de los factores anteriores determina si las moscas habitan una hoja enrollada: mientras esté un poco enrollada, es probable que estén allí y que no necesiten una cierta cantidad de materia acumulada para habitar una hoja. Además, la cantidad de artrópodos en una hoja no afecta la cantidad de materia acumulada. Por otro lado, sospeché que los fitotelmata efímeros de estas hojas enrolladas pueden servir como el hogar de larvas de *Stenomicra*, así que corté las bases de las hojas para poder buscar larvas bajo un microscopio. Encontré dos larvas de mosca en la materia acumulada en las hojas enrolladas, una de las cuales es *Stenomicra*. Con este hallazgo, es posible que las moscas *Stenomicra* que habitan estas hojas enrolladas completen su ciclo de vida completo dentro de ellas.

A common phenomenon in the insect world is the lack of information with regard to a single species, genus, or even whole families. One can sometimes find names and ranges of a species, yet find no pictures or description of the organism or their life history. While this is problematic at times, it means there still remains a whole host of fascinating insect biology to uncover.

The microbiomes within Zingiberales plants hold many understudied insects. This order of plants is common throughout Neotropics, especially in Costa Rican tropical forests (Kress, 1990). The temporal microbiomes that Zingiberales leaves provide are known to host many insects, especially Coleoptera, a majority of which are chrysomelids and ptiliids (Jalinsky, Wertemberger, Radocy, & Chaboo, 2014). *Stenomicra* is a genus of small, often yellowish flies of the family Periscelididae, who also inhabit these Zingiberales leaves. Unlike the beetles who reside in the same leaves, many *Stenomicra* have been left to this fate: they have names but little to no description of their life histories.

Out of the 34 extant described species, three of them live in Costa Rica (Freitas & Ale-Rocha, 2011). The little information that has been gathered about them suggests that most species may be associated with monocots, and the adult flies may be saprophagous or phytophagous (Gomes, Ale-Rocha, & Keppler, 2018; Merz & Rohacek, 2005). The adults of several species may be found on the surfaces of leaves, while the larva have only been found in aquatic or semi-aquatic environments within the phytotelmata of Bromeliaceae, Araceae, and Gramineae, Liliaceae, and Apiaceae; larvae of one species has been reported to eat culicid larvae (Campos, Gramajo, & Lizarralde de Grosso, 2010; Hamada, Thorp, & Rogers, 2018).

I observed *Stenomicra* at Quebrada Máquina at the Estación Biológica in Monteverde, Costa Rica. *Stenomicra* sp. adults live on *Heliconia* sp. (Heliconiaceae) and *Calathea* sp. (Marantaceae) leaf surfaces. They are identifiable by their black and yellow bodies, red eyes, and peculiar style of walking; they can run quickly in any direction in a crab-like fashion, always facing upwards. I noticed that some leaves caught more matter (rotting plant material, frass, dead insects) than others. The material inside appeared ideal for fly larvae and perhaps the adults too, and thus may influence whether or not they inhabit a leaf. Often, other arthropods like spiders, orthopterans, and chrysomelid beetles inhabited the leaves, and they likely contributed to some of the matter in the leaves with prey carcasses or frass. The stage of the rolled leaves is also a possible factor determining the presence of *Stenomicra*. I propose that the amount of matter found within these rolled leaves, the presence of other arthropods within the leaves that could contribute to the amount of matter, and the stage of the rolled leaf affect whether or not the *Stenomicra* flies inhabit them.

Many dipterans rely on aquatic environments for their larval stages, and larvae of 13 Diptera families are known to live in phytotelmata (Derraik & Heath, 2005). I believe that the ephemeral phytotelmata of these rolled leaves may serve as the larval home of *Stenomicra*, and they may feed on the matter that collects in these leaves. Phytotelmata refers to the water that impounds in plants, as in tree holes or plant axils; a classic example is the aquatic microcosms formed inside the leaf bases of bromeliad plants. *Stenomicra* in Zingiberales leaves could be able to complete their larval and pupal stages before the phytotelmata unrolls. This is interesting

given that the other families of plants in which larvae have been found have much more permanent phytotelmata.

MATERIALS & METHODS

I surveyed rolled leaves around Quebrada Máquina near Estación Biológica in Monteverde, Costa Rica. All samples were found around the creek where there is a high density of *Heliconia sp.* and *Calathea sp.*, with the exception of one plant, which I collected at the Monteverde Institute. *Stenomicra* flies are found in the young, rolled up leaves of these plants. For each leaf of each plant, I counted the number of adult flies present and then collected the bases of the rolled. Subsequently, I rated the amount of matter (dead insects, plant material, frass) inside the leaf on a scale of 0 to 3, zero being no matter and three being a lot. I also made a scale of 1 to 4 to rate the age of the leaf, with 1 being tightly rolled and 4 being a single curl (**Fig. 4**). I also counted the number of other arthropods (orthopterans, spiders, chrysomelids) in each rolled leaf because I wanted to see if their presence would contribute to the amount of matter in a rolled leaf.

To determine the species of *Stenomicra* living in these leaves, I collected three flies using and aspirator. I stored them in vials with 70% ethanol. To collect the contents inside the leaf, clipped off the base of the leaf entirely to keep any matter and water present contained with the sample. I sifted through the samples in petri dishes under a dissecting microscope to look for larvae.

RESULTS

My sample size was $n=20$ rolled leaves. There is no correlation between amount of leaf matter and abundance of flies in a rolled leaf ($R^2=0.022$; **Fig. 1**). Flies were most commonly found in leaves that were in stage 2 or 3, but the results from the one-way analysis of variance show that any degree of rolled leaf may have flies ($R^2=0.242$; **Fig. 2**). Out of all the mature unrolled leaves I counted, none had flies on them (**Table 1**). Twenty out of the twenty-three rolled leaves I found had flies living on them.

A regression analysis between the presence of other arthropods in the leaf (chrysomelids, spiders, orthopterans) and amount of matter in a leaf shows that the two variables are not related ($R^2=0.100$; **Fig. 3**).

I found two fly larvae within the phytotelmata of the leaves. I am certain that one is *Stenomicra* larva (species unknown, compare to pictures in Gomes et al., 2018). The other appears to be larva of another Diptera species.

TABLES & FIGURES

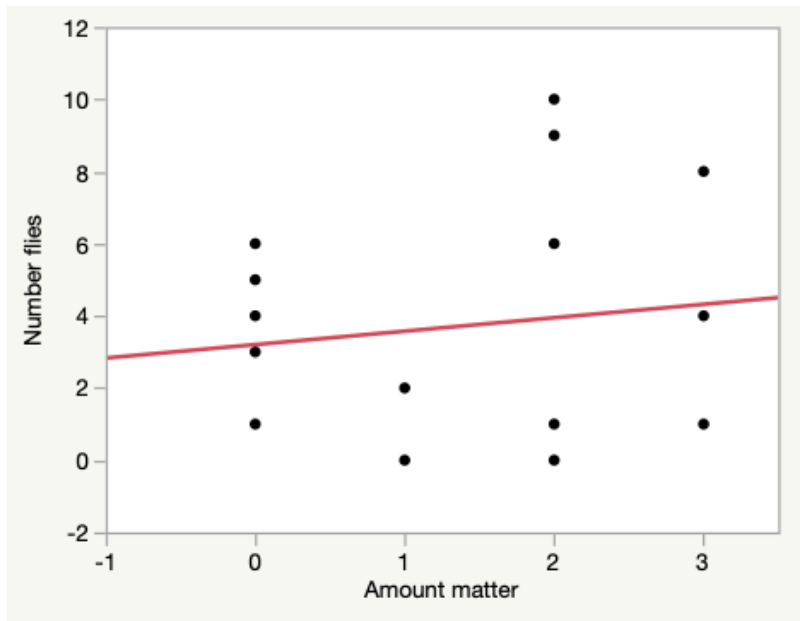


Figure 1: Number of flies versus amount of matter (rated on a scale of 0 to 3) present in each rolled leaf ($n=20$).

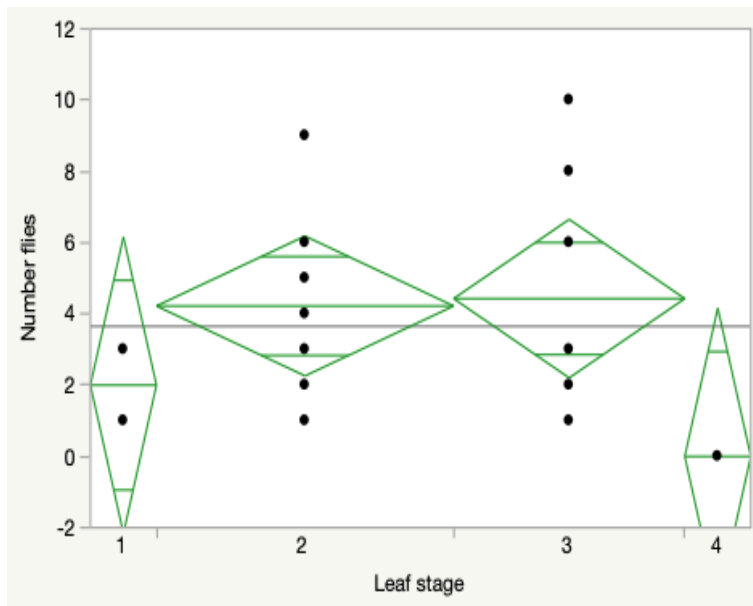


Figure 2: This graph shows that flies can be found on any stage of rolled leaf, with stages 2 and 3 exhibiting the most flies ($n=20$ rolled leaves).

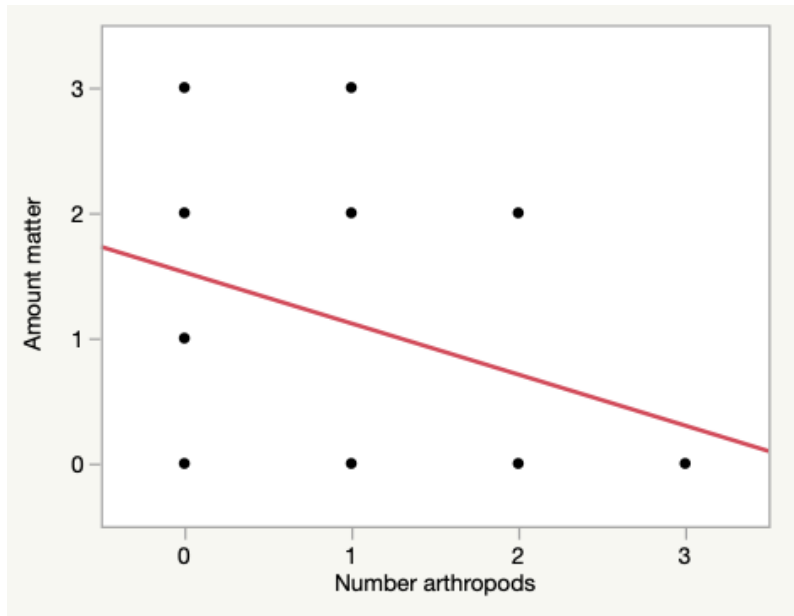


Figure 3: Amount of matter (rated on a scale of 0 to 3) versus number of arthropods in each rolled leaf ($n=20$).

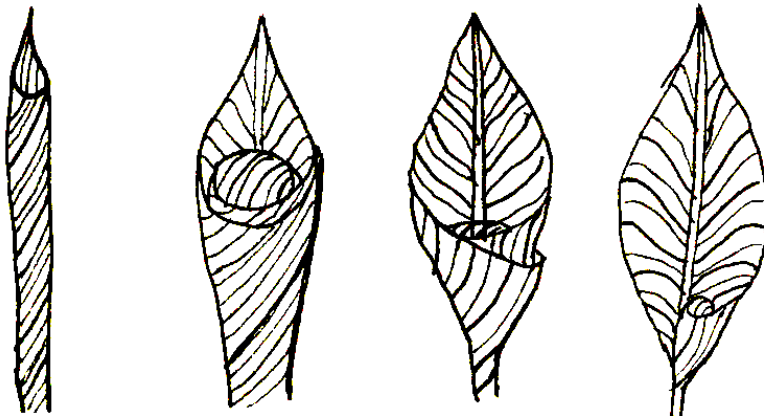


Figure 4: Stages of leaves, with stage 1 (left) being tightly rolled, and stage 4 (right) showing only a small curled edge.



Figures 5. **A)** shows a presumed female of one *Stenomicra* sp., **B)** shows a presumed male of *Stenomicra* sp.

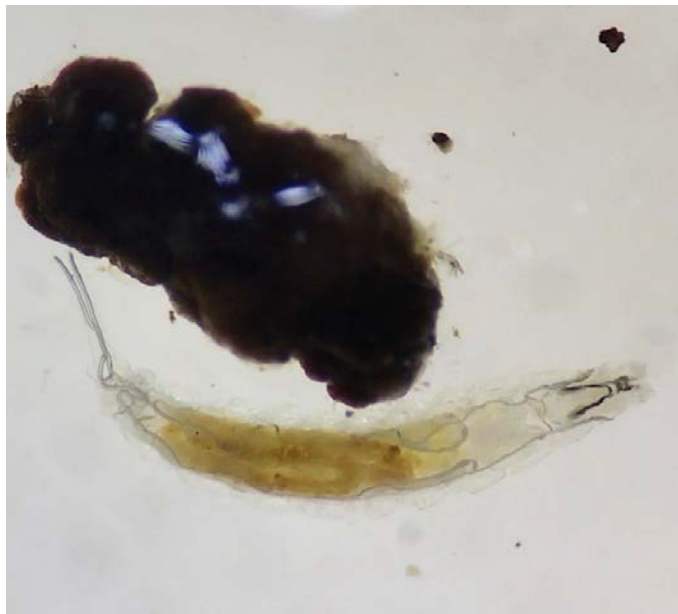


Figure 7: Larva of *Stenomicra* and the piece of matter it was found on and possibly feeding from.

Table 1: Incidence of flies on mature vs. rolled *Heliconia* sp. and *Calathea* sp. leaves.

Leaf Type	Total Mature Leaves	Mature Leaves with Adult Flies	Total Rolled Leaves	Rolled leaves with Adult Flies
<i>Heliconia</i> sp.	190	0	9	8
<i>Calathea</i> sp.	256	0	14	12

DISCUSSION

The incidence of flies does not increase with the amount of matter in a rolled leaf, contrary to what I predicted. I think it is possible that these adult flies are saprophagous and would therefore require the matter for nutrition. I found two fly larvae within the phytotelmata of the Zingiberales leaves, one I believe to be *Stenomicra*. The *Stenomicra* larva was found in a rolled *Calathea sp.* leaf. *Stenomicra* larvae are known to inhabit the aquatic or semi-aquatic environments of phytotelmata, but their inhabitation of phytotelmata in rolled *Calathea sp.* (Marantaceae) leaves has not been recorded (Gomes, Ale-Rocha, & Keppler, 2018). In addition, Diptera larvae have been primarily found in phytotelmata of *Heliconia* and *Calathea* bracts, not rolled leaves (Jalinsky et al., 2014). Upon initial observation under a dissecting microscope, the *Stenomicra* larva was atop a piece of wet, decaying matter. It was not clear if it was eating the matter, though it appeared that it might have been. The other unknown fly larva was found nestled in a leaf base among some matter and water in a rolled *Heliconia sp.* leaf. While observing the flies on the leaves, I could not determine what they were eating because of their small size. It may be possible that they suck up water and whatever microbial contents it may contain off the surface of the leaf, or they may be saprophagous; many would be deeper in the leaf where the matter collects, while a few would be higher up in the funnel. I did not observe any behaviors that would lead me to believe that they are phytophagous.

The flies reside on any stage of leaf, as long as it is rolled to some degree (**Fig. 2**). Often leaves that were not too tightly rolled and not too unfurled had the most flies. After clipping some tightly rolled leaves (stage 1) I noticed that they did not contain much debris and that there were many layers of rolled leaf, which I presume would make it difficult to have an accessible phytotelmata for the flies. If they are in fact using the phytotelmata of the leaves for larvae, it would make sense that less flies are on the extreme stages of leaves that either have inaccessible or absent phytotelmata. The temporal nature of *Heliconia sp.* and *Calathea sp.* rolled leaf phytotelmata would mean that the larvae would need to develop relatively quickly. It is possible that the larvae could achieve rapid development if food is abundant and climatic conditions are favorable (Lounibos, Frank, Machado-Allison, Ocanto, & Navarro, 1987).

Contrary to my prediction, the amount of matter in a leaf and number of arthropods in a leaf are unrelated. There would sometimes be one large cricket inside a leaf that would produce a lot of frass, resulting in high amount of matter for that leaf (this explains the distribution of many points in **Fig. 3**).

The intriguing life cycle of *Stenomicra* in Costa Rica is a bit closer to being decoded from this study. Now that a larva has been found in a rolled *Calathea sp.* leaf, it is possible that they may spend their entire life cycle in these ephemeral rolled leaves. Even though stage of leaf and amount of matter present in a leaf do not seem to influence whether or not they inhabit a leaf, we now know that it is possible to find them across a range of leaf stages and matter abundances. For further research, it may be interesting to find more of these larvae and attempt to rear them so that a complete record of their life cycle can be made. It would be interesting to see how much time is spent in the larval and pupal stages, and how this coordinates with the time it takes for a leaf to unfurl. Furthermore, the diets of the adults and larva are still largely unconfirmed, and would be exciting to uncover. Their mating habits would also be interesting to

study; I watched the males chase females across leaves, and watched the females flick them off frequently. On a couple of occasions, upon looking deep into the rolled leaves, I saw some pairs mating. The reason for their strange manner of running across leaf surfaces are yet to be understood as well.

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