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How Many More Flies?

April 2022 was a month of many surprises — all involving Dipterans (flies). The first was the article in *National Geographic* about the entomological adventures in Brazil by a group of experts led by Dr. Brian Brown of the Los Angeles Country Natural History Museum. While we had never met, I knew of him (but not the reverse) as the Museum's entomological curator and the boss of one of our long-ago interns: Lisa Gonzalez, who spent a month at Nectandra in 2005 studying insects and parasitic flies—Brian's area of expertise. In the *National Geographic*[1] article, his stunning photomicrographs revealed tiny Amazonian creatures (flies, bees, beetles, etc.) of unimaginable beauty. I was in awe, with a touch of envy, of the unsurpassed photographs and the exquisite insects.

Shortly after and out of the blue, I received a letter from the very same Brian Brown, inquiring if the Nectandra Preserve would host about 10 entomologists for a week of collecting in August. He heard of our cloud forest preserve via a colleague and has driven by our Nectandra gate many times. He wished to make contact and sent along reprints of his work in Costa Rica to provide the context and reason for his proposal. Of course I said yes! The opportunity to get a glimpse of Nectandra's dipteran diversity and to look over the shoulders of not one, but 10 experts was too good to pass up. In short order, Brian applied for the necessary collecting permits. We helped on the local arrangements for meals, accommodations and made plans to set up a rudimentary field laboratory for ten.

Fig. 1 Phorid fly *Pseudacteon* sp. USDA Agricultural Research Center. Wikimedia Commons

To ready for their visit, I boned up on the topic closest to Brian's heart — phorid flies — a subset of 4000 of the 160,000 known Dipterans. What I knew of flies in general would fit on a pinhead. About phorid flies, even less. They are the tiny, (0.4 - 6mm, ¼ in or less) humpback flies (Fig. 1) that skitter around detritus and decaying organic matters of all sorts (insect frass, carrion, dungs, dead animals, rotting vegetation, etc.). Many are parasites of an array of organisms, ranging from earthworms, snails, to soft-bodied pupae of insects, caterpillars or insect eggs etc. Half of all known phorid species are from the tropics.

Phorids may be minute in size, but their impacts on agriculturally important fauna and flora, plus their curiously diverse life-styles, make them noteworthy and often notorious. While some are general parasites for a broad range of hosts, most phorids are picky and prey on a single specific host. Below are just three examples of the fascinating and somewhat vampirish interactions between parasite-prey pairs. Bear in mind that there are thousands more of potentially and similarly strange Dipteran interchanges yet to be described.

Take for example the ant-decapitating fly (*Neodohrniphora curvinervis)* studied by Brian. The pressing objective of a gravid female is to locate food for the development and survival of her eggs. The food for her young is the head muscle and brain tissues of the leafcutter ant. The tiny gravid fly must seek out and locate her moving preys, not of any ant but only of *Atta cephalotes* foraging workers. Using undetermined cues for the task, she locates and swoops down on the ant column and brushes up to the targeted ant repeatedly, preferring one with head capsule larger than 1.6mm. In a swift second, she injects/deposits her single egg precisely on the ant's dorsal "neck" region and makes her escape before the victim and/or its fellow ants can fight back. In a successful ambush, the egg attaches, hatches, and enters the ant head. The maggot proceeds to evade the host's immune system while consuming its host from within. During the next 2 weeks, the host (superficially) goes about its business as usual while the fly larva fattens on its host's brain tissues to pupation. When ready to emerge, it exudes enzymes to digest the ant's head-to-thorax connective tissue. The head falls off. The fly exits and flies off to start a new cycle.

In a variation on the same theme, the fly *Apocephalus borealis* Brues parasitizes European honeybees, bumbles bees and wasps. In Fig 2A, the tiny *Apocephalus* fly (2-2.9mm) is inserting her eggs in the honeybee's dorsal abdomen.

1. https://www.nationalgeographic.co.uk/photographer/brian-brown

Fig 2A Female *Apocephalus borealis* fly ovipositing on abdomen honey bee *Apis mellifera.* Photo by Christopher Quock. Wikimedia Commons.

Fig 2B Larvae of *A. borealis* exiting infested bee. Photo by John Hafernik. Wikimedia Commons

Parasitized honeybees, in a highly abnormal behavior, abandon their hives at night, fly toward light, move in puzzling circles and die with multiple fly larvae emerging from the bee's neck (Fig 2B). Of the California beehives surveyed, 77% of the Californian hives are fly infested. While current evidence points to a multi-causal (pesticide, fungal & viral infection) explanation for the honey bee Colony Collapse Disorder, *Apocephalus* infestation undoubtedly contributes to the decline of a $215 billion pollinator industry worldwide.

My third example affects us humans. The *Dermatobia hominus* botfly (15 mm) lay eggs on female mosquitos. The eggs, still growing inside the mosquito, are injected into humans (or cattle) through the skin during a mosquito blood meal on the mammalian host. The eggs hatch and the maggots mature within 10 weeks in a subdermal cavity, breathing through a hole in the host skin. Without revealing the details, I invite my readers to watch a most informative (and somewhat disturbing) short video on *Dermatobia*[2] by Piotr Naskrecki (world renowned biologist and photographer. Botfly myiasis is a fairly common condition in both humans and cattle in the tropics. I recall the experience of one of our Canadian interns who, after a side trip to Belize, went home to Canada with three discrete red lesions, which he immediately recognized as botfly myiasis. Unfortunately, his primary physician did not agree with the self-diagnosis and prescribed antibiotics for his skin condition. Days later, unable to withstand the intense itchy sensation of the wiggling maggots, he searched for a more receptive physician and found relief only after the larvae were surgically removed.

I now turn to Brian's more recent work. From his entomological training and start in Canada, he seemed to have slept and breathed Dipterans!

His list of field collecting trips read like an atlas — Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, Guyana, New Zealand, Peru, Taiwan, Thailand, to name a few. On the way, he collaborated with a small army of international Dipteran experts. Under his authorship, the number of new species mounted, detailed descriptions of known phorids flourished, all leading to inescapable revisions of subgroups and eventually their reclassification. In going through the phorid literature, I have the distinct impression, as an inexpert reader, that in the last two decades the whole field of Dipteran biology has literally exploded. It is in a state of great flux. Scientists are continuously surprised by their sheer biodiversity, in species, morphology, ecology and behavior.

In the academic paper he sent me, Brian began his introduction by imagining himself in Galileo's shoes when the latter first trained his eyes through the newly invented telescope. Galileo was astounded by the huge number of hitherto never seen stars. Overwhelmed, he also realized that he could only study a small fraction of them in his lifetime. Similarly, Brian also rued about phorids at the end of his paper (2018) titled *Remarkable fly (Diptera) diversity in a patch of Costa Rican cloud forest: Why inventory is a vital science,* Zootaxa 4401: p53-90.

The paper described the results of an intense, concerted effort of his team of international expert entomologists over a single year, using continuous trapping with Malaise traps combined with regular, monthly 3-day discontinuous sampling using 9 other supplementary trapping methods. During that year, 15 expert members also "bioblitzed" for a week using all of the trapping methods at once, combined with field identification. In this study, unlike other investigations of this type, the authors tried to overcome two major challenges — inherent selection bias using discontinuous and spotty sampling, plus incomplete identification (especially when faced with a high number of specimens). To that end, they sampled continuously 24/7 for a year. The team examined *all* trapped Dipteran specimens and identified them to the highest known taxanomical level. This was accomplished by a team of 6 technologists who locally processed/sorted the contents of the traps, then prepared (pinned or slide mounted) the specimens to be shipped to overseas experts for examinations. The final identification was the work of 59 co-authors with expertise covering all of the 76 known families of Central American Dipterans.

2. (https://thesmallermajority.com/2015/01/12/dermatobia-redux/)

The key question of this study was: How many species of Dipterans are there in a 4 Ha (10 A) cloud forest located in Zurquí, Costa Rica? The location was selected for logistic reasons and not for prior knowledge of its biodiversity. Of a total of 53,000 dipteran specimens trapped and examined at this tiny 4 Ha, the answer was an astounding 4332 species! Put in perspective, the country with the world's highest known number of dipterans, Colombia, had 3523 known species recorded. Other collateral information from the study involved the effectiveness of trapping methods, species turnover, species density, among others. Brian's proposal for Nectandra (~75 km NW of Zurquí), in the next three years is simply to extend the range of Dipteran exploration.

By early August 2022, we at Nectandra were ready and waiting for the arrival of the ten entomologists at our gate. At the appointed time a rented van pulled up. Ten eager-faced individuals spilled out of the packed van. Each stretched for a few seconds, unloaded their gear. They lined up for a quick handshake, and cheerfully gave their names. Before I could even register their faces and introduce myself, they had turned around, quietly picked up their nets or traps, marched toward the several trails and disappeared into the forest, without so much as a map of the place! In mere minutes, I was standing alone with the van and driver. It had to be the briefest introduction to 10 strangers I have ever experienced!

Over the course of the next five days, it slowly dawned on me that for these men and women, collecting came first, before food, weather, rest and sleep, in that order. On arrival each day, the group would scatter into the forest, return to the visitor center for lunch, only to disappear again into the clouds and forest until overtaken by rain, darkness or exhaustion. At that point, if there were any daylight left, I would catch them quietly working at their dissecting microscopes, sifting through all the specimens trapped, photographing them with an impressive array of camera gear for macrophotography, or processing them for later identification. After they returned to their lodging for dinner, work on their specimens continued afterward on the dining table until the wee hours.

On the third morning, one of our garden staff came to me deeply puzzled. He reported the presence of meters-long tracks left by an unknown animal, unseen in his eighteen years at Nectandra, characterized by upturned and freshly chipped logs all along our trails! His bewilderment cleared only after I explained that one of the entomologists specialized in ant phorids (parasites of mature ants) as well as ant parasitoids (parasites of immature stages). Many of our tropical ant species nest inside rotting logs, hence the coarsely pulverized logs to get to both the colonies of ants, and ant larvae.

As a group, they worked efficiently, quietly and required little assistance. Few things could distract their attention. As the person in charge of their nutritional sustenance while they were at Nectandra, my attempts in slowing them down for lunch only succeeded by extending the meals with tasty desserts! In the opposite vein, back at the B&B one evening, the host accidentally dropped the words "flies and compost" while conversing. Instantly, the room cleared. He triggered a stampede toward the garden at his disclosure of flies around the compost pile!

We at Nectandra were humbled by Brian and his team. They are committed to an enormously important but gargantuan task of cataloguing our planet Earth. Their work has further exposed our collective colossal ignorance of the living world.

The core mission of our organization, as our revered fellow founder Alvaro Ugalde put it "is to save the biodiversity of our planet so that the biologists can biologize". To see the "biologizing" in action, done with enthusiasm, care and utter devotion, is a reward unto itself. We dedicate this newsletter to them in appreciation. *— The editor—*

2022 Highlights

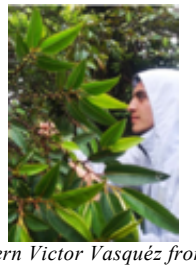
Our two staff members, Randall Varela (education and community outreach) and Manrique Esquivel (biologist), have been literally "embedded" among our partner communities as part of the eco-loan program. They spend fulltime the last 7 years providing technical, advisory and other hands-on assistance on reforestation and water issues of all manners to the communities.

On the Forest Front — *reported by Manrique Esquivel*

In 2022 we had a lot of collaboration from young volunteers from Switzerland, Germany and England (photos below) and interns from a local technical school as part of their graduation requirements.

Volunteer Ash Cooper from England

Volunteer Laura Popp from Germany



Intern Victor Vasquéz from Piedades Sur, CR

In the nursery operated by Liga CUENCA (a league of 8 eco-loan recipient ASADAS) produced 630 trees of 21 different species delivered to different community partners and individuals. Most of the tree species selected were pioneer trees with high capacity to survive in pastures and in difficult ecological conditions. These trees provide important ecosystem services such as soil formation, water production, pollination, seed dispersal, among others. In this video (https://www.youtube.com/watch?v=3xGYRxVJHJ8) you can see the main phases carried out for the production of these trees.

August 2022 marked the 50th anniversary of Coopealfaroruiz, a local electricity cooperative and community partner of the Nectandra Institute which acquired a 36-acre property in 2013 for forest and water conservation located in the El Chayote Protection Zone. Throughout this process, pioneer trees were planted, maintained, some as windbreakers to promote natural regeneration. What a different panorama (photos below) between 2013 compared to today on the property!

A video[3] documenting the symbolic trees planting as part of the 50th anniversary celebration can be viewed on Facebook.

Fig 4. The two photos taken from the same reference point shows the extent of regeneration during 9 years. In contrast, the two adjacent farms not under NI eco-loans, at left and right on top of the slope remained as pastures.

3 https://www.facebook.com/ZTVZarcero/videos/5270841293042322/

On the Education and Community Front

— reported by Randall Varela

As our forest under the eco-loans is growing, so is our successful work with the communities. From a single ASADAS in 2006, we have now an ecoloan network composed of more than 3 dozen nearby communities. In the past few years, NI has made an effort to introduce our conservation and work model to other regions in the country, as well as to members in the National Confederation of Water Users. This national network hares a common need — to provide clean potable water to every rural household. There is much to learn from each other. Within this network, NI encourages and coordinates regional workshops, training, exchanges of information of all topics associated with water and water delivery.

MAR — Several smaller ASADAS (with <700 water meters) in our region visited the much larger ASADAS San Roque de Grecia (>1500 water meters, not an NI partner) to see and learn about their new facilities and new water telemetry installations.

JULY — 2022 was an exceptionally wet year, with hurricane Ian and several periods of sustained rain. Liga CUENCA initiated an Emergency Assistance Technical Team that monitored, evaluated and was in readiness to deal with possible damage to drinking water systems among the partner communities. Training sessions for plumbers and managerial members within the member ASADAS were organized to deal with weather as well as seismic related emergencies.

AUG to DEC — Participation in many meetings to exchanges information and experiences among many of the 1300 water within our The from and

community associations and beyond watershed. topics ranged tariffs, regulations, improvement protection of water resources.