Using DNA-barcoded Malaise trap samples to measure impact of a geothermal energy project on the biodiversity of a Costa Rican old-growth rain forest

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Abstract: We report one year (2013–2014) of biomonitoring an insect community in a tropical old-growth rain forest, during construction of an industrial-level geothermal electricity project. This is the first-year reaction by the species-rich insect biodiversity; six subsequent years are being analyzed now. The site is on the margin of a UNESCO Natural World Heritage Site, Área de Conservación Guanacaste (ACG), in northwestern Costa Rica. This biomonitoring is part of Costa Rica’s ongoing efforts to sustainably retain its wild biodiversity through biodivelopmental integration with its societies. Essential tools are geothermal engineering needs, entomological knowledge, insect species-rich forest, government–NGO integration, common sense, DNA barcoding for species-level identification, and Malaise traps. This research is tailored for integration with its society at the product level. We combine an academic view with on-site engineering decisions. This biomonitoring requires alpha-level DNA barcoding combined with centuries of morphology-based entomological taxonomy and ecology. Not all desired insect community analyses are performed; they are for data from subsequent years combined with this year. We provide enough analysis to be used by both guilds now. This biomonitoring has shown, for the first year, that the geothermal project impacts only the biodiversity within a zone less than 50 m from the project margin.

Key words: DNA barcode, geothermal project, Costa Rica, biomonitoring with insects, ICE/ACG/SINAC/MINAE/GDFCF.
Introduction

Philosophical

The detailed reactions of the biodiversity of whole terrestrial communities of tropical wild eukaryotic biodiversity, to industrial-scale perturbations, have not yet become a major topic of research interest for the academic or commercial community. The social and technical focus has been on the avoidance or repulsion of such perturbations. The notable exceptions are for single species that are endangered, charismatic, disease carriers, major pests, or have other species-level traits of special interest to humans. The causes for this neglect of a potentially huge research area by the science community are (i) centuries of absent capabilities for global database (big data) synthesis, and (ii) human inability to simultaneously identify the thousands of insect species in a whole-community analysis.

Widely used electronic data management (e.g., Ratnasingham and Hebert 2007, 2013) and DNA barcodes (Hebert et al. 2003, 2004; Holloway 2006; Ratnasingham and Hebert 2013; Janzen and Hallwachs 2016; Miller et al. 2016) now allow avoiding both of these impediments. Here, we report on the use of electronic species-level biodiversity data management and DNA barcoding of large samples of tropical insects to deliberately and simultaneously (i) apply biomonitoring with insects of a species-rich and relatively unperturbed old-growth tropical forest that has been point-source impacted by an industrial geothermal project, and (ii) lightly touch on basic research questions about a tropical insect community.

Throughout the terrestrial tropics there are a plethora of industrial perturbations to well-established forests that are generated by roads, mining, hydroelectric dams, river capture, light pollution, water pollution, atmospheric pollution, pesticides, logging, agriculture, and tourism (at the least). Examination of the impacts of these perturbations is almost entirely based on the believed or actual impacts on populations or individuals of single species of vertebrates that are usually charismatic, large, and threatened with extinction by some criterion. These species generally constitute less than 0.1% of the biodiversity present. Measurements generally ignore whole phyla and orders of biodiversity, and tens of thousands of species (e.g., fungi, nematodes, mites, insects, spiders, non-timber and juvenile plants, as well as the microbes that ride on or in them). A basic reason for neglect of this 99.9% of wild tropical terrestrial biodiversity is that the species are not only almost totally undescribed by science, but also that it has been generally impossible to identify them and to communicate to the lay community about them as species and as identified individuals, for whatever purpose. Perhaps worst of all, they are viewed as unimportant to humans and their domesticates, in great part due to human ignorance of the details of their lives, and due to human desires to live distant from the wild world that birthed our genomes.

This paper is meant to outline some of the many pragmatic steps that occur in the melding of academic basic biodiversity research with the pragmatics of biomonitoring an industrial geothermal perturbation, as a module within Costa Rica’s new ongoing BioAlfa project to know and accommodate its own national wild biodiversity (Janzen and Hallwachs 2019a, 2019b), which is in turn a module within BIOSCAN’s efforts to DNA barcode the globe (Hebert 2015; www.ibol.org). This report is neither a review of the literature about the topic, nor anything like the detailed scientific scrutiny deserved by the massive amount of data produced and continuing to be produced. Rather, it is meant as an introduction and exploration of an example of melding pragmatic social desires for production with the curiosity-driven research that is standard within the academic community.

Pragmatic

Many items or facts strategically mentioned throughout this introduction and analysis are details of the kind that are not normally reported in either scientific or engineering analyses, or both. They are included, and many more omitted, because they are the kinds of guild-to-guild accommodations that were used to create a win-win situation for two well-entrenched and well-appreciated sectors of society that traditionally have quite different goals and measures of success. This report deliberately mixes them together because that is the way they occur in reality.

Mots-clés : codes-barres à l’ADN, projet de géothermie, Costa Rica, biosurveillance d’insectes, ICE/ACG/SINAC/MINAE/GDFCF.
The portion of the biomonitoring of the geothermal project called Pailas II in northwestern Costa Rica (ICE Group Description 2019) originated at the end of nearly two decades (1997–2013) of neighborhood overlap between two long-term projects: (1) very basic biodiversity science and action as biodiversity inventory of Costa Rica in general, and specifically the inventory of the project’s adjacent Area de Conservacion Guanacaste (Janzen and Hallwachs 2016, 2019a, 2019b), and (2) the quite pragmatic event of government harvest of a geothermal resource 2–3 km underneath the old-growth mid-elevation complex tropical rain forest of the ACG UNESCO Natural World Heritage Site (http://www.acguanacaste.ac.cr) and its contained Parque Nacional Rincon de la Vieja. This several thousand-hectare mid-elevation forest (Supplementary data, File S1) is divided roughly in half by the boundary between the long-established Parque Nacional Rincon de la Vieja in Sector Pailas of Área de Conservación Guanacaste (ACG) of MINAE (Ministerio del Ambiente y Energia) (http://www.acguanacaste.ac.cr). The relevant lower half of this mid-elevation rain forest is likewise government-owned land, but it was purchased by a different agency of MINAE, the Instituto Nacional de Electricidad (ICE). ICE is a National Energy Company operating as a government agency (ICE Group Description 2019). This land is explicitly developed as the geothermal project known as Pailas I and Pailas II (ICE Group Description 2019). The specific study site discussed here is drilling platform PL12 of Pailas II, and its surrounding old-growth forest. Its margin is about 60 m from the ACG boundary (Figs. 1–3, 5).

This geothermal project appears to have the potential to be highly destructive to the adjacent ACG UNESCO Natural World Heritage Site and to any old-growth mid-elevation rain–dry forest in its vicinity. Equally complex, the Pailas II PL12 site lies exactly in the intergrade between dry forest and rain forest. This kind of intergrade tropical forest has been almost totally ignored by biodiversity studies, and contains portions of the biota of both of the larger ecosystems as well as a unique biota (e.g., Janzen et al. 2017).

When the PL12 geothermal site was initiated in 2013, it had all the ingredients of a classical Greenpeace versus commercial industry conflict as a small war about important conserved and conservable old-growth forest between two opposing government agencies in the same Ministry. The various anticipated conflicts, and their classical non-solutions prior to 2013, are not discussed here, as they would require another yet more biopolitical report of equal length and not involve DNA barcoding and its application as as part of the technical solution to the conflict.

### History, materials, and methods

On 23 August 2013, several middle-management ICE engineers and planners visited ACG’s Área Administrativa...
tive to explicitly describe their plans for the geothermal development of Pailas II on the ACG boundary (the yellow line in Fig. 1). This visit was prompted by ICE’s middle-management having heard and absorbed a five-year litany from the ACG staff (www.acguanacaste.ac.cr) and that of the Guanacaste Dry Forest Conservation Fund (GDFCF; www.gdfcf.org) as to “why don’t we work together to find a definable minimally damaging solution for the harvest of this well-known geothermal resource?”. On 23 August, ICE was feeling administratively and biopolitically free to approach ACG, with GDFCF (http://www.gdfcf.org) as a collaborating NGO, in hopes of a response to this conservation question.

At the end of the ICE presentation, GDFCF asked ACG’s Director if it would be permissible to introject a pointed observation. He responded, “OK, if you want to”. GDFCF asked the ICE presenter for permission to use the ICE PowerPoint slides to explain what ACG/GDFCF would do if it were their geothermal project, a project that we all knew the authors of this report would see made sense to do. The presenter in turn asked the most senior ICE person, who turned out to be the Chief Engineer of Pailas II, and they had their conversation. The Chief Engineer replied in effect “OK, discuss, but this is all unofficial”. GDFCF took the presentation and said “imagine that NSF had just given GDFCF/ACG a US$2 million grant to ask a question. “What is the reaction of the biodiversity of a complex tropical old-growth rain forest to having a one-lane access road constructed through it, and an accompanying 1–2 ha drilling platform cut out of that same forest?”. GDFCF proposed a novel project to use lines of Malaise traps (Fig. 5; Supplementary data, File S2) for at least a year and hopefully more, to document the reaction by the tens of thousands of species of flying insects in that forest. A trap will be placed at the road margin, at 50 and 150 m distant inside the forest, and then this three-trap line repeated from two points on the margin of the drilling platform, for a total of nine Malaise traps. The traps would be serviced on the same exact day every week of the first year by the same parataxonomist (Janzen and Hallwachs 2011) and hopefully continue for years after. Every insect captured would be DNA barcoded by the Centre for Biodiversity Genomics at the University of Guelph (http://ibol.org), just as ACG/GDFCF has been doing for years to inventory its own adjacent forests inside ACG (Janzen and Hallwachs 2016). ACG/ICE/GDFCF would then jointly begin to address how has the road and drilling platform perturbed this insect biodiversity and at what distances from the perturbation. In this case, a baseline year of traps running pre-perturbation was not possible, but the traps were set up at the very beginning of the perturbation to the forest.

This proposal was followed by more discussion among the ICE staff. ICE engineering sites are generally off limits to non-ICE personnel and to work-in-progress analysis by others. ICE is frequently attacked by NGOs for real or imagined environmental damage done to preserved or conservable wild ecosystems, and generally attempts to follow the traditional national Environmental Assessment rules from MINAE’s SETENA (Secretaria Tecnica Nacional Ambiental) for engineering projects. ICE is viewed with envy by many other government entities that have far less funding (in part because ICE produces a huge portion of Costa Rica’s electricity). ICE is largely staffed and budgeted with professionals and workers who know very little about wild biodiversity, and who do quite large unintentional damage, thereby attracting the ire of conservationists. ICE is conspicuously a different government agency than SINAC (the National System of Conservation Areas) but a dependency of the same government ministry, MINAE.

At the end of the discussion, ICE took a leap of faith and said “OK” to the challenge. The ACG/GDFCF reply was, “then you need to take us immediately to the forest and show us exactly where the access road will be, and where the PL12 drilling/extraction platform will be”. Two days later, ICE did. And the engineering budgeteer then said quite memorably, “but how can we know how to budget it when we will not know how many insects will be caught”? This conundrum was resolved with a guess of US$60 000 per trap-worth of barcoding per year averaged over the nine traps; the trap became the practical unit, rather than species or specimens. After the end of the year, financing was available for analysis of only seven traps (the contents of the other two remain in the freezer), but that was vastly better than none, from both a basic science and an engineering viewpoint.

The members of the ACG/GDFCF Parataxonomist Program (Janzen and Hallwachs 2011; https://www.acguanacaste.ac.cr/programa-de-parataxonomos) were then asked to set the traps as soon as the one-lane roadway was bulldozed and the platform margins were likewise chain-sawed and bulldozed out of the forest.

Why only nine Malaise traps? Because a student researcher from the Centre for Biodiversity Genomics (CBG; http://ibol.org) had visited ACG the year before and left nine Malaise traps in the storeroom. Why 0, 50, and 150 m? Guesswork and a start towards other distances for later planning. Why Malaise traps? Because they could be set and attended by a parataxonomist or other outsider with no formal biological background, or by an ICE engineering staff member, and because there were freezers in ACG in which to store the weekly samples in 95% ethanol. And because CBG had already established the proto-BioScan global Malaise trap sampling scheme (Hebert 2015; http://ibol.org) that was quite experienced at Sanger-sequencing mass Malaise trap samples to build the global biodiversity Eukaryota barcode library in BOLD (http://www.boldsystems.org). ACG/GDFCF had already contributed three Malaise trap-year samples to the
proto-BioScan (http://ibol.org) effort for ACG bioliteracy and bioinventory (Janzen and Hallwachs 2016).

The weekly samples were accumulated individually in 300 mL glass bottles in −20 °C flat freezers for a year, then shipped in neoprene bottles by DHL to the CBG at the University of Guelph. This was done under MINAE and CONAGEBIO permits for their collection and export, explicitly for DNA sequencing for their individual barcodes and for basic inventory research. This required formal permission from ICE, the legal owner of all terrain being trapped, except traps #1 and #6 that are positioned just inside ACG (Fig. 5). In the CBG the insects were individually Sanger sequenced, the extracts and cadavers stored for later taxonomy and (or) Costa Rica’s CONAGEBIO contracted genome exploration. The barcodes and their collateral are stored on BOLD as global public domain, as authorized by the Government of Costa Rica (e.g., Government of Costa Rica 2019). Further taxonomic refinement of specimen description, and at times identification, is in continuous process (e.g., Janzen and Hallwachs 2016; Espinosa et al. 2017; Fleming et al. 2018; Arias-Penna et al. 2019; Burns et al. 2008; Fernandez-Triana et al. 2014, 2015). This process is integrated with the ongoing DNA barcoding of all of the biota of ACG, and now, the cross-society BioAlfa program to DNA barcode all of the million eukaryote species estimated to live in Costa Rica, for national bioliteracy and for integration of their conservation with their society through non-damaging biodvelopment (Janzen and Hallwachs 2019a). All project data will become progressively more available to any global user as its processing gains taxonomic and organizational maturity over the next 2–5 years.

But what of barcoding costs? The initial PL12 engineering site preparation and development to production cost roughly US$22 000 000. These funds were part of an US$800 000 000 overseas development loan from JICA (Japan International Cooperation Agency). ACG/GDFCF assumed that it would be important for JICA and ICE not to have an environmental black eye on their international environmental balance sheet. They would therefore be willing to fund the comparatively tiny estimated costs of DNA barcoding the biomonitoring results, especially if voluntary rather than forced by NGO attack. After negotiations, JICA provided the projected US$420 000 to meet the direct cost for the DNA barcoding by CBG for about 145 000 specimens (Fig. 6). ACG/GDFCF initially provided all else pro-bono to the project. The CBG provided its BOLD web site analytical services pro-bono. Small costs (ETOH, bottles, two freezers, rental car fuel, parataxonimist, and PI time) were GDFCF investments in the project, though they were later partly reimbursed by JICA as a lump sum consultant fee.

JICA, their consultants, and middle-management ICE inspected the entire project and found it to fit the JICA concept of a SAPI. A SAPI has been explained as “Projects are prepared with assumptions in the preparation stage that they sometimes face unpredictable events and issues in the implementation stage. In such circumstances, JICA organizes a scheme called Special Assistance for Project Implementation (SAPI) on a grant basis as a countermeasure to seek feasible solutions for a smooth implementation of the project.” (M. Hasegawa, JICA, personal communication 15 December 2019). GDFCF received the funding and passed it on to CBG with no overhead charges.

A SAPI and the ACG/GDFCF/ICE involvement contained the first year of environmental planning and inspection for the major ICE project underway, along with standard SETENA (as mentioned previously) requirements. A wide variety of visitor inspections and educational events then introduced entomological trapping, barcode sequencing, taxonomy, and basic ecology through and around the geothermal project and its staff, gradually leading to its insertion into the much larger complexity of the engineering requirements and actions. This was coupled with small but highly significant site adjustments (e.g., open gates through protective fencing, reduced lighting, one lane access road, later platform adjustments considering the actual traps, unobstructed educational and research access by foreigners and Costa Ricans, NGOs, government agencies, and planners). It became an on-site and in-symposia display as part of Costa Rican efforts to integrate necessary environmental perturbations into its green country vision. Its data, because the insects were individually barcoded, are a major starting point on building the BioAlfa national DNA barcode library (Janzen and Hallwachs 2019a), and ground-truthing for future changes in the insect community. This paper is one of these efforts. It displays biodiversity facts of interest to biodiversity ecologists, engineers, and biopoliticians, but simultaneously is about research on the socioeconomics of blending industry perturbations with conservation of tropical wildlands, something that ACG and GDFCF have attempted to develop since 1985 as an ACG founding principle (e.g., Janzen 1986a, 1986b, 2000; Janzen and Hallwachs 2016, 2019a, 2019b).

Results

Partial GDFCF and CBG data analysis for the first year of weekly Malaise trapping at drilling platform (plazoleta) PL12 of the Pailas II ICE geothermal project (November 2013 to November 2014)

The direct technical subtopics of this report, interspersed appropriately rather than strung together in one package, are the following. All require that the individual insects can be known by their DNA barcodes and by their dates of capture by the Malaise traps in space and time, as performed by the CBG. In most cases for each, the Order, Family, BIN, and barcode were machine-obtained from BOLD, but where that failed, morphological identifications were occasionally enlisted for family-level identification. BINs, as defined with DNA barcoding...
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(Ratnasingham and Hebert 2013), are treated as synonymous with species, though other more detailed studies (D.H.J. and W.H., personal communication; see Janzen et al. 2009; Janzen and Hallwachs 2016) show that about 10% of them contain two or more closely related species in the same genus. The outcome is that, for example, 1000 BINs from a Malaise trap sample is actually represents about 1100 species.

Introduction to data

Context is everything when it comes to measuring environmental variables such as perturbation. The context of the PL12 biomonitoring is a clear example. As described above, this experimental pilot project to biomonitor the PL12 geothermal site with Malaise traps for flying insects was voluntarily initiated on 23 August 2013 by middle management of ACG/GDFCF and ICE in four different yet overlapping contexts:

1. Can two different and often competing National Government agencies (SINAC, ICE) and a conservation NGO (GDFCF) collaborate in an actual project to begin to understand, ameliorate, and minimize the environmental damage that unavoidably occurs when portions of an industrial project such as a geothermal development project are inserted into an intact forest of high conservation value, next to the boundary of the sensitive and formally protected Área de Conservación Guanacaste (Fig. 2, a UNESCO Natural World Heritage Site, National Park, and NGO land purchased for conservation) (http://www.acguanacaste.ac.cr)?

2. Can DNA barcoding of tens of thousands of undescribed insects solve a portion of the seemingly insoluble problem of rapid individual and species discriminations (identifications) within massive samples of extraordinarily species-rich tropical insects? If so, this will allow the use of Malaise traps (or any other trapping method) as biomonitor as if they were weather stations monitoring rainfall, with each captured insect being analogous to a drop of rain with its date and temperature. If so, Malaise traps and minimally trained staff can generate a baseline for presence and changes in a species-rich biodiverse community.

3. Is it possible to document a degree of perturbation, and the location of that perturbation, generated by the insertion of a geothermal project into a protected forest? If so, it allows consideration of whether (i) such a project is to be allowed by national conservation desires, (ii) there is an appropriate form and amount of mitigation, and (iii) if the classical engineering structure traditionally used for such a project can be modified to minimize its impact.

4. These considerations allow generic guidelines, with site-specific adjustments, to be developed for the use of Malaise traps (and other trapping devices) as biomonitorers for industrial projects that have cause to be sensitive to their impact on their adjacent biodiverse ecosystems, but lack an available community of people professional at biomonitoring. This report is an ad hoc preamble to such guidelines.

The PL12 project has begun to explore all of these topics for the first year of the PL12 biomonitoring project that began in August 2013 (hard data beginning in November 2013), and during the year of SAPI data analysis (March 2016 – March 2017). All four of these topics are, simultaneously, a work in progress and will continue to be for years as more comparable case studies occur in other sites, and as the biomonitoring of PL12 with these Malaise trap sites is continued through at least 2020.

As a key underlying process to all of this report, we distinguish here between the traditional kind of “Assessment”—the “A” in EIA (Environmental Impact Assessment)—and biomonitoring as being conducted for PL12 with Malaise traps. Standard EIAs are usually conducted at approximately one point in time and place, classically near the beginning of the project and to obtain a government permit to proceed. The goal tends to be to detect the presence of threatened species or other charismatic environmental resources, with hopes to avoid or mitigate. The PL12 goal of biomonitoring with insects or other easily monitored biological variables is to notice changes so as to respond to them as the geothermal extraction rolls forward for decades, and to plan other similar integrations of conservation with industry. A camera trap photograph of the jaguar that lives near PL12, or its footprints, during the years to come, tells us only that it is present and nothing of its reactions. A year of Malaise trapping sets a baseline (Fig. 20). The year 2013 begins to document biodiversity changes in response to specific events (onset of drilling, night lights, distance from the site, exhaust fumes, comparison with seasonal changes, consequences of trap placement, site exposure to wind, sun and direct rain, climate change, and restoration). However, such analyses do require entomological, ecological, and taxonomic knowledge following the computerized trapping and barcode analyses, a prime opportunity for collaborations between field biology and industry.

This biomonitoring is tracking ecosystem-level real or suspected perturbations of biodiversity by recording ecosystem or species changes in time and space in relation to the specific perturbation, hopefully throughout the life of the project. It is one of many kinds of biodevelopment towards integration with the societies that own the site. The ACG/GDFCF collaboration with ICE/JICA is based on the concept of at least six total years of biomonitoring and hopefully long after that, both for the benefit of the geothermal project itself (PL12 in Pailas II) and for JICA’s goal of “improving geothermal biomonitoring strategies” as a supplementary project goal for other places and other countries. Biomonitoring is therefore viewed here as useful for (i) predicting perturbations by as-yet-
uninitiated other projects, (ii) guiding initially unanticipated measures for damage control or mitigation over time, (iii) learning about unimagined ecological processes that take years to become evident, and (iv) gathering baseline and raw data for quite esoteric basic science such as building DNA barcode libraries for a multitude of other social users including the taxasphere. Equally important as an underlying tenet, biomonitoring may document tracking/invasion/extinction of particular species or interactions, and the use of tracked variables (in this case, the presence of thousands of species of insects) as an indicator of other changes such as global climate change. Over time, the Malaise trapping discussed here will serve these various purposes, though as with any trapping technique, only a subset of the total site biodiversity is obtained with Malaise trapping. How much of the total will only emerge through comparative sampling by other methods from the same forests. Currently, the other methods are light-trapping and litter sampling, both underway since 2013, but with no funding for analysis of the results in the freezer.

Collaborative insertion into the ACG forest and neighbor

This ACG forest as a whole (Sector Pailas and Sector Santa Maria + Pailas II) has long been viewed as having great conservation importance. The portion of forest in ACG’s Sector Pailas, immediately north of Pailas II (Fig. 2), was the first piece of private land that Sr. Alvaro Ugalde (RIP), the founder/developer of the Costa Rican National Park System, fund-raised for and purchased in 1971.

In 2011 it was realized that this ecosystem of mid-elevation rain forest is environmentally important for two other reasons, one largely social and the other biological. First, it was named PreCafetal (Supplementary...
data, File S1) because it is the kind of forest (soil, elevation, climate) that Costa Rica has cleared very thoroughly for 150 years of coffee plantations, a crop indirectly responsible for much of Costa Rica’s current environmental awareness. Indeed, this portion of ACG is the only readily accessible large area of this kind of forest ecosystem remaining in Costa Rica, in or outside of a national park. This intermediate-elevation forest is therefore a biological national antique, a reminder of what once was, and what was cleared, in the establishment of the Costa Rican middle class through the developing and developed coffee industry, with all its subsequent consequences. There are still feral coffee trees growing in this forest, escaped from small backyard coffee plantings made by early colonists in Sector Pailas and adjacent Sector Santa Maria (Supplementary data, File S1).

Second, in Costa Rican Zonas de Vida terminology (= Holdridge Life Zones, see Google), the protected Sector Pailas potential geothermal site is Bosque Muy Húmedo Premontano (BHMP) and Pailas Dos itself is Bosque Muy Húmedo Transición a Premontano (BMHTP). The border between these two Life Zones cuts right through the PL12 drilling platform in Fig. 1 (see Fig. 3). Within ACG’s Sector Pailas and Sector Santa Maria there are 4525 ha of BMHP and 1654 of BMHTP (Waldy Medina, ACG cartographer, personal communication). However, three years of inventory of the caterpillars, moths, and butterflies by GDFCF/ACG parataxonomists at PL12 indicates that PL12 also lies on the mid-elevation fuzzy boundary between the large ecosystem of Tropical Rain Forest (which once covered lowland Caribbean Costa Rica) and Tropical Dry Forest (which once covered most of the lowland Pacific side of Guanacaste Province).

These blurry intergrades between major Life Zones and ecosystem categories are precisely the kinds of habitats that are heavily deforested, farmed, and ranched elsewhere in Costa Rica and in the tropics in general. They are nearly eliminated and very poorly conserved. They and their biodiversity occupy a severely threatened habitat type, one that is generally ignored when worrying about “saving the rain forest” or “saving the dry forest”; each of these two is its own distinctive ecosystem, and the intergrade between them largely ignored (Janzen 1986a, 1986b). Ecosystem margins are small, fragile, and easily obliterated, yet occupied by both their own peculiar biodiversity (e.g., Janzen et al. 2017) and by the fluctuating edges of distributions of widespread species. Such places are classical species generators in the evolutionary dynamics of landscapes. This renders their biomonitoring, when perturbed locally by industry or globally by climate change, to be of particular importance.

Unfortunately, and unavoidably, both Pailas II of ICE and Sector Pailas of ACG are perched on top of a very large geothermal energy resource. A geothermal development drills down into this hot water, uses it to run a generator plant, and injects it back into its source. It is a

Fig. 3. The site of PL12 in Pailas II is the white dot in this Holdridge Life Zone map. The black margin is that of Parque Nacional Rincon de la Vieja (Sector Pailas and Sector Santa Maria of ACG, and therefore is the margin of this UNESCO Natural World Heritage Site as well). [Image: Waldy Medina from original coordinates.]
comparatively clean perturbation that can be tightly controlled. The question then becomes can ACG/GDFCF and ICE work together in this project collaboration, instead of having the classical Greenpeace-type confrontation of environmental conservation versus industrial development over a conspicuously valuable natural resource? The goal is, following a general principle of disciplined decentralization by both SINAC and ICE, for ACG and ICE to be allowed to determine among themselves how to get that geothermal resource out from underneath Sector Pailas with minimal ecological perturbation, and appropriate compensation for whatever damage does occur. Biomonitoring of Pailas II is part of the mechanics of the two government agencies, ACG/ SINC and ICE, working together. It also has the potential of limiting the damage to the Pailas II forest to that which is truly necessary for the extraction of the geothermal resource lying beneath it.

Forgetting the various false starts beginning in 1997, the short answer to this key collaboration question, since August 2013, is “yes”. A Greenpeace situation has already been firmly avoided. ACG and ICE are working very closely together, and show all indications of continuing to do so. This is actually the first and most important characteristic of successful biomonitoring. It is more important and higher priority than even the technical aspects of Malaise trapping, barcoding, and resulting academic findings. Since August 2013, in great part supported by JICA (Japan International Cooperation Agency) and overseen by the ERM (Tokyo) consulting firm, GDFCF, ACG, and ICE have collaborated (Fig. 4) in the philosophical and technical biomonitoring of PL12 of Pailas II, and in its associated biopolitics and policy evolution. These are continuing through 2020 and beyond. ICE has now invited ACG/GDFCF to actively participate in the planning of their next geothermal project (Borinquen, well to the west of PL12 but likewise adjacent to ACG) before it has even begun.

The next step in biopolitical demonstration of success is exemplified by the side panel presented by Costa Rica’s CONAGEBIO, MINAE, SINAC, and ACG at COP13 of the Convention for Biological Diversity (CBD) on 8 December 2016 in Cancun, Mexico (“Mainstreaming biodiversity in development: A case of geothermal development in Guanacaste, Costa Rica”). This presentation was financed by a collage of different Costa Rican national and international agencies, each thereby supporting their own agendas as well as the collaborative agenda for PL12, and beginning the evolution of guidelines as an export product for other geothermal projects. This is an international example of ABS, with the benefit being biomonitoring know-how and its accompanying biopolitics. This kind of benefit stands out as an example of the kind of know-how benefit that can be shared without creating ownership conflicts among the sharing partners.
At some time in the close to distant future, the government of Costa Rica may be so eager for clean, reliable, and comparatively cheap geothermal electricity to meet its ever-expanding demand, that the major geothermal resource under this portion of the ACG Natural World Heritage Site will be irresistible to mass development by the government. At that time, there may not be people and groups who would invest the serious time, funds, and biopolitical energy to induce collaboration or chain themselves to trees as can occur with Greenpeace-type confrontations. Should this day come, it is very directly anticipated that the ongoing ACG/ICE Pailas geothermal experience that began in August 2013 will be directly valuable in helping to minimize community-level environmental social perturbation, both technically and biopolitically. Should this day come, it is very directly anticipated that the ongoing ACG/ICE Pailas geothermal experience that began in August 2013 will be directly valuable in helping to minimize community-level environmental social perturbation, both technically and biopolitically. In short, the Pailas Dos biomonitoring of PL12 and its access road (see Google image of the site, Fig. 5), is an experiment as if it were happening inside ACG’s Sector Pailas on a newly placed drilling platform with its access road.

While it will be well into 2020 before the next big steps in the ACG/ICE collaboration around PL12 will become better defined, it has been recommended by ICE and JICA that the nine traps biomonitoring PL12, and the following five years of samples in the freezer, have their analysis continued, though funds for this have yet to be sourced. Additionally, traps #1, #2, and #3 may remain as the continuation of the beginning of long-term biomonitoring of PL12, now that a baseline has been established. Simultaneously, in the second year ICE expanded the PL12 platform by about 0.5 ha in one direction (north) and added a second 1 ha static processing plant in the other direction (south), both of which desire further biomonitoring. The cost of all biomonitoring of the kind described here will be legitimately included in the normal anticipatory and continuation budgeting for a geothermal project in or adjacent to a conserved wildland, just as are included administration, studies, plans, bridges, roads drilling, generation, and marketing.

Ongoing continued comparisons of the Malaise trap results from PL12 with ongoing ACG internal and national biomonitoring by various methods and BioAlfa national biodiversity inventory (Janzen and Hallwachs 2019a) will also occur, irrespective of decisions made about continuation of PL12 biomonitoring. This is because as the years go by, the increasing taxonomic refinement of Malaise trap barcoding and taxonomic results (i) continue to increase their value for better comparisons locally, nationally, and internationally, (ii) build the regional public barcode library for all users through the internet, and (iii) because more comparative DNA barcoding results are constantly being recorded everywhere. All of this contributes to global perception and understanding of biodiversity. In a few years an insect that is currently known only from ACG or PL12 may also become known (by its DNA barcode) from Mexico, Guatemala, or Colombia. The same applies to birds, fungi, and weeds. Every time another site anywhere is biomonitored with Malaise traps or other trapping, the scientific networking of Central (and even South) America is increased, by virtue of the collaborative pooling of this

Fig. 5. Drilling platform PL12 (~1.4 ha) in March 2014 (after one year of existence). The nearly invisible one-lane entrance road enters the platform from the lower left under the forest canopy. The immediately adjacent old-growth forest appears to be, and largely is, intact. The nine Malaise traps were placed, and have been maintained, as numbered in October–November 2013. The yellow straight line approximates the formal boundary between the UNESCO Natural World Heritage Site Área de Conservación Guanacaste, and Costa Rica’s National Electric Company (ICE). [Image: The base image is from Google Earth, March 2014 downloaded by Alex Smith, with indicators added by D.H.J.]
taxonomic information (DNA barcodes with their associated collateral taxonomic names) for all users in all social sectors via the BOLD integrative engine on the internet in the Centre for Biodiversity Genomics at the University of Guelph at http://www.boldsystems.org; http://ibol.org. See below for an example with *Simulium* blackflies (“bocones”) that feed on humans (Fig. 8).

DNA barcoding solves identification problems

Malaise traps (Fig. 9) were invented by taxonomists to collect insects for taxonomy or calculating trappable species-richness of a site, rather than for biomonitoring (e.g., Darling and Packer 1988; Flowers and Hanson 2003; Gaston et al. 1996; Brown 2005; Sjoberg and Teal 2014). They are one among many kinds of insect traps used by taxonomists to collect, but have generally been excluded from tropical biomonitoring because of the daunting task of even beginning to morphologically sort the massive number of trapped specimens to species or higher taxa that they collect. Equally daunting, it is obvious that they catch only a large but unknown subset of the insect species in a particular site, as is the case with any trapping system. Additionally, it is generally not the case that the taxonomy-focused collector is stationed in one tropical place for multiple years and interested in faithfully sampling week after week. Finally, taxonomists are usually interested in sorting through Malaise trap samples to find the particular (generally new) species of interest largely to them in their focal taxa, and are usually not interested in the total set of thousands of other species that are caught.

DNA barcoding each individual insect in an entire Malaise trap sample of many thousands of insects by Sanger sequencing or other sequencing methods (e.g., Srivathsan et al. 2019) is, however, a technically superb way of jumping over these barriers. It converts a Malaise trap into an actual biomonitoring device, just as a weather station is a rain-monitoring device. The cost per insect for all of the processing is currently still high. It averaged about US$2.89 per insect (average of US$60 000 per trap for seven traps) for the mass Sanger sequencing and analysis performed in 2016 by the CBG, for the 144 994 insects from the first year of seven Malaise traps from PL12 (Fig. 6). However, this price is substantially cheaper than the US$10–20 per insect cost when the ACG biodiversity inventory began barcoding in 2004. The price today for processing plus sequencing is US$1 per Malaise-trapped insect (P.D.N. Hebert, personal communication; CBG contracts for US$2 million with ACG/GDFCF and the Walder Foundation https://news.uoguelph.ca/2020/01/u-of-gs-centre-for-biodiversity-genomics-awarded-4-million-to-catalogue-life-in-costa-rica/). The biomonitoring samples in the CBG receive a variety of discounts for bulk processing efficiency and because they are simultaneously a contribution to the global insect barcode public service library accumulating in BOLD (http://biodiversitygenomics.net, http://www.boldsystems.org) as part of global BioScan (www.ibol.org), which services Costa Rica and Central America as well. To date,
Costa Rica has contributed at least 45,000 species of insect barcodes to BOLD, out of the perhaps 650,000 species of insects estimated by the BioAlfa project to live in Costa Rica (Janzen and Hallwachs 2019a).

Fig. 7. Of these 11,385 BINs, 99.93% were successfully assigned to Family by their barcodes without further effort. As the years and decades pass, the numbers assigned to taxonomically recognized groups at the species level will approach 100% as (i) the number of barcodes from other places in BOLD increases, (ii) taxonomists describe and revise their respective taxa of interest and these names are added to BOLD, and (iii) more rapid protocols for scientific descriptions evolve (as they currently are, e.g., Meierotto et al. 2019). A greater level of taxonomic completion is not required for the first set of conclusions reached here and now, though further taxonomic refinement will permit much more use of the data for both pragmatic geothermal biomonitoring, climate change monitoring, and barcoded biodiversity research. [Image: Screen shot created by multiple coauthors from CBG.]

<table>
<thead>
<tr>
<th>Order</th>
<th>BINs Identified to Family</th>
<th>BINs Identified to Subfamily</th>
<th>BINs Identified to Genus</th>
<th>BINs Identified to Species</th>
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Grand Total 11,385 11,377 2,720 1,644 459

99.93% 23.89% 14.44% 4.03%

The success per insect DNA barcoded and assignment to a BIN code in the 2013–2014 PL12 year, averaged 90.5% across the 144,994 specimens in the first synchronized year of 21 November 2013 to 14 November 2014 (Fig. 6). Of
these, there are 11 385 BINs. A BIN approximates one species, with a BIN containing the different individuals with the same barcode (Ratnasingham and Hebert 2013). It is equivalent to a morphologically assigned grouping that assigns individuals to the same species based on their morphological similarity. As with a morphology-based species name, a BIN may contain more than one similar (so-called cryptic) species, each separated by more than simply a ~2% or less barcode difference (e.g., Janzen et al. 2017; Hebert et al. 2004).

About 99% of the insects from PL12 were successfully machine-assigned to insect Order and Family by their barcodes (Fig. 7). With 15 years of experience working with DNA barcodes of 500 000+ ACG insects identified through their nearest neighbor joining phenograms (NJ trees) that are correlated with natural history traits (food plant, host parasitoid, microgeographic location, season, and elevation) (e.g., Janzen and Hallwachs 2016), leads us to estimate that the eventual number of species that will be found to be in this one-year large sample from PL12 will be about 10% more than the number of BINs, or about 12 000 species. This is because experience with ACG samples have shown that about 10% of BINs contain two or even three cryptic similar species that can only be distinguished by very careful morphological examination, correlation with ecological traits, or a deeper genetic analysis (e.g., Burns et al. 2007, 2008; Smith et al. 2006, 2007; Janzen et al. 2017). Of the 11 species of ACG Astraptes butterflies found to be hiding inside one scientific name (Hebert et al. 2004), six have now been found to reside in the same BIN.

Most of the unsuccessfully barcoded specimens could likely be DNA barcoded with a second try. However, this would require a more detailed laboratory treatment at a consequently doubled or greater expense. Contaminated specimens could equally be re-barcoded. All specimens have been retained as DNA barcode vouchers at the CBG and a photograph logged for at least one intact specimen from each BIN. These photographs are also available in BOLD by searching for individual voucher codes or taxa names, or BIN codes.

All of this barcoding of mass samples, and therefore national biodiversity inventory (Janzen and Hallwachs 2019a), increases the demand and opportunity for taxonomic efforts that are coordinated with the current new possibilities for collecting, curating, and correlating the data offered by DNA barcoding, by BOLD, and by mass collecting techniques. It should be emphasized that the 4.03% figure to the right in Fig. 7 is the percent identified with a real scientific name that is already in BOLD from other sampling and taxonomizing in ACG. Such an identification means that the PL12 insect barcode matches one of an identified insect in BOLD, many of which are ACG specimens laboriously described and therefore scientifically identified during the past 35 years. The percentage in this column is low in large part because the great majority of the captured insects belong to new, undescribed species, and do not yet have scientific names. But ALL of the 11 385 BINs have effectively been identified with a unique BIN code. This almost-taxonomically legal interim name is directly analogous to the human-friendly sorting of pinned museum specimens into species hypotheses by what they look like and giving them an interim nickname. In this context, it is not surprising to find that a BIN can be found to contain multiple species when its barcoded members are matched against other morphological and ecological characteristics (e.g., Janzen et al. 2017).

This allows for national and international comparisons, once its conspecifics from other places are also registered in BOLD. Equally, this species-level (or sub-genus level) taxonomic tag allows each species presence or absence to be recorded per trap and per date (even though it currently has no scientific name), now and over future years.

There is a specific example of these inter-country comparisons. Anyone working in PL12 in the daytime, from visitors to engineering crews, notices immediately that there are often large numbers of small human-biting blackflies or “bocones” (Simulium spp., Simuliidae) on the grounds of the drilling platform in the rainy season. These small flies (Fig. 8) are major blood-sucking pests for mammals throughout ecosystems rich in freshwater streams, where their larvae filter out plankton and debris as the water runs past them. The Malaise traps were expected to capture Simuliidae, and indeed, six species were captured at PL12 (including two undescribed species) and identified by their barcodes through BOLD. Because these flies are also carriers of human disease (Onchocerciasis or “river blindness”) their taxonomy has recently been revised for Central America (Hernandez-Triana et al. 2015) and in BOLD.

This identification in turn allowed the beginning scrutiny, through Google, of each of the described species, with a relevant research result from the biomonitoring. On the vegetation-free platform, it is notable that by moving just a few meters from the edge into the shade of the forest, there are no more human-biting blackflies. However, people standing just a few meters away in the open are frequently swatting them as they bite (Fig. 8). One species identified by BOLD by barcoding, Simulium tarsatum, was found very rarely in four out of six deep forest traps, but very commonly in the trap on the platform edge (#3). However, the literature reports that S. tarsatum feeds on birds and other small animals, and not on humans; the eDNA analysis of their gut contents showed that they feed on humans as well (E. Zakharov, personal communication, Fig. 8). On the other hand, the most abundant of all, Simulium jobbinsi, was caught only in the platform edge trap (#3); S. jobbinsi is well known to be a species of blackfly that feeds largely on humans. However, their eDNA gut analysis showed that they also feed on many other species of wild vertebrates (birds,
bats, and small mammals). It is also a transmitter of Africa-origin Onchocerciasis by the nematode causing river blindness. In other words, the rapid barcoding of the biomonitoring samples allowed understanding of the local distribution of closely related species of flies that are potential disease transmitters, and to highlight that they are therefore a potential health problem for the ICE staff working at the drilling platform, but much less of a threat in the adjacent deeply shady forest understory. It is conceivable that this kind of detailed information will gradually and eventually become available for most, if not all, of the species whose presence has now been documented for the PL12 forest and its drilling platform.

Easily 90% of the species captured by the PL12 biomonitoring Malaise traps are undescribed (= "new") species. As such, they are not accompanied by the species-level taxonomic literature that is characteristic of extratropical species. As a single example of this shortage of collateral information and classical scientific names, to date ACG has collected, by rearing and Malaise traps, about 1100 species of Microgastrinae wasps (very small Braconidae that are parasitoids of caterpillars); prior to their taxonomizing by the ACG/GDFCF inventory of ACG, only about 3% were described species (e.g., Fernandez-Triana et al. 2014, 2015; Arias-Penna et al. 2019).

There is no question as to whether DNA barcoding works for the insects captured in the Malaise traps. It has been demonstrated in ACG since 2003 that it works to identify species and discover new ones (Hebert et al. 2004; Janzen et al. 2009; Janzen and Hallwachs 2016), albeit requiring understanding of its quirks and cautions as is the case with any science or engineering protocol. The PL12 specimens to date have been barcoded by Sanger sequencing. This PCR-based method normally generates barcodes of 500–658 base pair lengths. These lengths have proven to be of very high quality for identification and species discovery for the more than 48 000 species and 500 000 specimens of ACG insects barcoded so far (e.g., Burns et al. 2008; Janzen and Hallwachs 2016; Miller et al. 2016; Smith et al. 2006, 2007; Fernandez-Triana et al. 2014, 2015).

Specific technical comparisons

**Compare traps #1, #6, and #9 as representative of deep undisturbed forest understory**

Traps #1, #6, and #9 were in the shady old-growth forest understory ~150 m distant from the edge of the

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**Fig. 8.** The presence of species of Simuliidae (blackflies or “bocones”) in the seven biomonitoring Malaise traps at PL12 (aerial view on the right). The species figured here, *Simulium jobbinsi*, is erroneously believed to feed largely on humans, and rapidly draws blood, as shown a few seconds later on the right when crushed. However, *S. jobbinsi* gut eDNA showed that it feeds on birds, bats, rodents, and peccaries as well as humans. Despite howler, spider, and capuchin monkeys being normal density in this forest, none tested positive for their blood. Note the capture numbers in trap #3 versus trap #4. [Image: Multiple composite by D.H.J.]
drilling platform PL12 and margin of its access road (Fig. 5). Their positions and distance from the platform were selected, and traps installed, in October 2013, immediately following opening and clearing of the one-lane access road and ~1.5 ha drilling platform. Changes in subsequent years for PL12 will be monitored from this same biodiversity baseline. The traps have been run (and replaced) through 2020, but further samples remained frozen at −20 °C, awaiting funding for processing (which is why this report touches only on the first year of biomonitoring).

The distance of 150 m from the perturbation was chosen because experiences as a tropical field entomologist intimately familiar with these ACG forests and their insects (e.g., Janzen 1973a, 1973b and subsequent decades) suggest that there will be no immediate impact of the drilling platform and road at this distance from them. Indeed, none was indicated by any of the trapping results (Figs. 10, 11). The perturbation generated no change in numbers of insect species throughout the year; they did change as expected during the normal dry season – rainy season cycle: low density in the January–May long dry season, a late May – early June mild peak with the first rains, and a gradual increase during the remainder of the year as adults emerge from the eggs and larvae started at the beginning of the rains and their cooler temperatures. Simultaneously, the additional perturbation of drilling (late February–August) is not evident in the results (Figs. 10, 11). See Supplementary data File S3 for a yet more complex but incomplete statistical analysis of this first year.

If the understory forest Malaise trapping were to have been done for only a few months, the striking seasonal changes in the site could easily have been confused with changes caused by the platform perturbation and drilling. Second, only continued Malaise trapping can determine if the platform margin itself will become a source of species and biomass contamination of the adjacent forest interior, though it did not in the first year. Whether it will, is dependent on many other variables. As expected, the graph of species richness of captures during the long dry season (January to May) was relatively flat and stable, followed by the expected increase of captures with the beginning of the rainy season in May (Figs. 10–11, 20–21). This was followed by the low rainfall (and sunny days) of the August short dry season (and the
time when many species are in a non-flying egg or larval stage), and then a steady increase of adults through the remainder of the rainy season (Figs. 10–11, 20–21). Trap #9 showed the greatest capture of species after the heavy rains. From late February 2014 to August 2014, the platform drilling (lights, noise, people, vehicles) were present (as indicated by the blue bar in Figs. 10–11, 20–21). There was no visible effect of the site preparation and drilling activity on the usual reaction by the insect community to the first rains of the year. However, a comparison of the two subsequent years of trapping from these three deep forest traps is required to confirm this observation’s persistence (to be published when funding is available).

Were these three traps redundant on each other, and therefore just one of them would have conveyed the same message? This question assumes, however, the risk that such a single sample trap would not be destroyed by a tree fall, landslide, wild animal, or vandalism. The patterns of species captured by each individual trap and their sum (Fig. 6), convey the same general message and constitute three deep forest understory baselines for subsequent years (and see Supplementary data File S3 for a complex statistical analysis that determines that they are not significantly different from each other). However, without their essentially identical results for this first year, there would be no way to begin to know what is a normal result for this deep forest understory insect community, for comparison with results closer to the platform edges. The other option would be a year of Malaise trapping prior to the beginning of this project, but engineering socio-administration did not allow that. Furthermore, the annual climate in that pre-project year could easily be different from the installation year.

The number of BINs in common among the three traps tells a very different story — only 13% of the BINs captured were caught by all three traps (Fig. 12). If only one trap had been used, the number of BINs would have been 41%–56% of these trappable BINs (by all three traps pooled). If two traps had been used, then about 70%–84% of the trappable BINs would have been captured. It is not possible to estimate the total trappable fauna from these data, since the BIN/trap curve (Fig. 11) is still rising sharply after progressively pooling all three deep forest traps, no matter in which order the traps are pooled.

The trappable insect biota at PL12 is sourced from three quite different microhabitats. First, there are the resident species in the shady understory, many of which have an ecology that is the opposite of heliophiles. Very many of the family most commonly captured, Cecidomyiidae, are in this category, and strikingly did not vary in proportion of species among all seven traps. Sec-
ond there are those that live in the (insolated, windy, dry) canopy above and are caught at ground level as short-term visitors (e.g., to oviposit where their larvae live in the litter or on low plants) or as waifs. Third, there is the light rain of species represented by single specimens (transients, waifs, migrants, lost, wide-rangers) that arrive on the canopy of the forest, but are derived from the ocean of insect populations and communities in the agroscape that abuts the ragged edge of the Pailas II forest, only 4–8 km distant. These species would normally be carrion or prey for the resident forest inhabitants. That they are captured by this study is the accident of them flying into the Malaise trap in their passage from arrival to being consumed. The Diabrotica beetles in Fig. 16 are classic examples of such agroscape species. This category of species can only be recognized through knowing them and their natural history in their home agroscape. Their numbers and seasonality within the insect rain will change in response to microclimates and perturbations in the agroscape, and the distance of that agroscape from the forest being inventoried. A currently unknowable but significant fraction of the species and numbers of the insects in traps #1, #6, and #9 (and #2 and #7), are probably such waifs (which of course may colonize the platform edge). With subsequent years of study of the insect fauna of the PL12 area (as well as of ACG as a whole), many of these will become known for their ecology.

It is notable that 65% of the 11,385 BINs in all seven Malaise traps have been caught by only one trap in this first year (see Fig. 6). There are at least two different (but simultaneously possible) ecological sources for such singleton records.

First, the BIN that is unique to a trap may be represented by only a single specimen captured during the year among all the traps, and therefore can occur in only one trap. Detailed studies of the ACG insect community for the past 35 years (Janzen and Hallwachs 2016) have indicated that in any given year, and often for consecutive decades, a very high proportion (perhaps as many as 75%) of the species of insects indigenous to a site occur naturally at very low density per species, and at even lower densities of trappable specimens. They are simply rare by extra-tropical trapping standards and expecta-
tions, but at normal abundance by tropical standards and expectations. It is no surprise to capture only one specimen of one of them in a trap during a one-year cycle. When there are a very large number of such species, sampling such a community structure by any method yields a very large number of singletons for a species.

Second, as mentioned above, there are two sources of very low density trappable specimens that come from the outside. The first is that visitors or waifs from the canopy community, even for species that are quite abundant in the canopy, may well contribute only one or very few individuals of canopy-resident species to a trap in a given year. The second is that the above-mentioned rain of insects blown or flying from the agroscape will contain many species at very low density in the specimen rain even if they are common in the agroscape.

On the other hand, whenever considering overlap among or between traps, if the singletons are ignored (since by definition a singleton cannot occur in two or more traps), the degree of overlap among the three deep forest traps becomes slightly more intense, moving from 13% to 18% (Figs. 12-13).

In summary, each of the three deep forest traps tells the same seasonality story and appears to be insensitive to the presence of the drilling platform and road, during the first year of the project. However, if the biomonitoring is directed at any particular species and whether it is influenced by the perturbations, a single trap would at best be capturing (monitoring) about half of the BINs in the pool of 4499 trappable BINs, and two traps pooled would capture about 75% of the BINs in the trappable pool. This speaks poorly to the concept of indicator species but is relevant to indicator communities.

At the Ordinal level, all three deep forest traps tell approximately the same story about the insects captured. As is the case with all ACG and PL12 Malaise trapping, by far the most abundant BINs are flies (Diptera or moscas) (Fig. 14), followed by beetles (Coleoptera), wasps and ants (Hymenoptera or avispas), small moths (Lepidoptera or mariposas), and then lesser numbers of bugs (Hemiptera or chinches).

By comparing Figs. 12 and 13 it is obvious that the overlap among the three traps is similarly low, with or without the singletons excluded. Equally, comparing Figs. 12 and 13 shows that singletons make up a large portion of the species captured by a given trap, while the overlaps among the traps with the total are almost identical when singletons are excluded (Fig. 12).

Turning to family-level taxonomic content of the individual three traps, or to all three of them pooled, in all cases they are extremely species-rich (Fig. 15). Cecidomyiidae flies are by far the most abundant BINs. It is tempting to wonder if biomonitoring just flies would effectively mirror the changes in the entire insect community. This question is provoked by the pragmatic desire by non-entomological users to wish for indicator species. At present, not nearly enough is known about the natural history of these animals to even think about finding indicator species (and, indicators of what, exactly?), or even wonder how representative (and, of what, exactly?) such species might be. It can, however, be concluded that the margin (e.g., trap #3) is rich in species
that are not normal or usual inhabitants of the forest understory. The margin is, in its biotic content and physical traits, analogous to a narrow edge of a pasture, roadside, old field, landslide, or insolated riverbank (Fig. 16).

Eventually, looking backwards and laterally through many years of Malaise trap samples from PL12, there will emerge some correlations between perturbations, seasons, and climate change. A few specific taxonomic groups or patterns will emerge as specific indicators of environmental point source and (or) widespread change in Pailas II forests. As for traps #1, #6, and #9, on the level of insect Order, the traps generated essentially identical results with respect to proportions of total numbers of species, and numbers of species of major taxa per week and overall. They are, therefore, a tri-replicate of what is normal for the forest understory at 150 m from the platform perturbation, both at the end of a year and during the year.

It is likely that as many as 10 more understory traps would have delivered the same overall results in this same year. Equally, for traps #1, #6, and #9, at the Family level, the result is about the same as that for the Order level. Each of the three deep forest traps is representative of the trappable insect biodiversity of the deep forest understory (along with its insect rain of currently unknowable sources from the canopy far above and the adjacent agroscape), in terms of numbers of BINs per week and how that parameter behaved for the first year. Since each of the three are the same as each other, and the same as trap #2 (Fig. 5), we conclude that the platform and its drilling have had no effect at all on the biodiversity of the forest at a distance of 50–150 m (and

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**Fig. 14.** Ordinal-level species composition of the trapped content of the three deep forest traps (#1, #6, #9). The shaded trap in the image is #9. The fly is a representative Cecidomyiidae (gall fly), the members of which make up half or more of the species of Diptera captured in PL12, and are numerically dominant in all seven traps. The galls on a grapevine are typical extratropical cecidomyiid galls that have a fly larva inside of each. However, foliage, flower, and fruit galls (e.g., Gagne 1978, Woodley and Janzen 1995) are very rare in this forest; the enormous number of species of Cecidomyiidae must have larvae with a different ecology, such as fungus-feeding and predation. [Image: CBG coauthors, raw data from study.]
further) during this first year. Current ongoing analyses of the first and second years of traps #5 and #8 will reject or reinforce this conclusion.

Whether this is also the case at the level of species behavior remains to be determined. It cannot be determined until when, years from now, the biology or the classical taxonomy of these species is sufficiently well known to be able to attribute natural history traits to most of the species captured (parasite, herbivore, mutualist, migrant, hyperparasite, mimic, and predator). However, that scientific information base is not required to initially address the question of whether the drilling platform or its drilling activity have impacted the adjacent forest understory at 50–150 m or more from the platform and road.

Compare traps #3 and #4 as margins of the drilling platform, and #7 as road edge

As has been noted previously, traps #3 and #4 recorded enormously different numbers of species of insects throughout the year, though the seasonal pattern of trapping is the same for both (Figs. 10–11, 17–21). The cause is that trap #3 serendipitously had its collecting bottle at the trap end pointed towards the sun of the open platform and trap #4 had its collecting bottle pointed towards the deep forest interior shade (and just marginally within that shade immediately adjacent). This happened because taxonomically inclined entomologists, such as ourselves, who normally sample with Malaise traps for taxonomy, usually put the traps in the full open or the full shade. No thought was given to the peculiarities of trapping exactly on an edge. This variable was not considered in the initial positioning of traps #3 and #4, and it has not been considered in any of the published papers describing Malaise trapping (e.g., Darling and Packer 1988). Most trapped light-loving insects escape upwards and are attracted toward light; shade-loving insects escape upwards and toward shade. We suspect that nocturnal fliers will be the same, but they use air temperature and moisture content to orient instead of sunlight. The upward direction of attempted escape is anticipated by the design of the roof of the trap, which slopes upwards towards the entrance to the collecting bottle (see Fig. 9).

Trap #7 (and #4) gave the same basic results as the other three traps that are far from the perturbation in the deep shade, as well as trap #2 which was only 50 m from the edge of the platform (and within sight of it through the forest understory). While the vicinity of trap #7 received a very heavy dose of dry season insecticidal road dust on its surrounding foliage, the trap itself was
actually heavily shaded by the nearly intact tall forest canopy on both sides of the one-lane road and therefore in the forest understory ecosystem. Trap #7 showed no significant perturbation effect of the presence of the road during this first year of trapping. While a complex statistical analysis appeared to locate the traps #7 and #4 results somewhat marginal to the cluster of traps #1, #6, #9, and #2, it will require analysis of years 2 and 3 to gain enough of a sample size to know if this is within or without the normal range of variability of these forest edge traps. The road dust unambiguously eliminated foliage-feeding insects (caterpillars, beetles, and bugs), as evidenced by the greenhouse-perfect condition of the foliage near trap #7 (no feeding damage). However, it is not surprising that flying insects of the forest understory (passing through) continued to be caught by trap #7 (and #4). It also needs to be emphasized that the one-lane road itself eliminated a small portion of forest habitat near trap #7 (just as is the case with the platform next to trap #4), which again emphasizes the different kind of perturbation (even if shaded) that it received in the first year. Finally, trap #7, as did #4, had its collecting bottle pointed into the shade of the forest, rather than into the slightly more insolated road.

The detailed statistical analysis of traps #4 and #7 showed a slight difference (more species of Hymenoptera and Lepidoptera) from traps #1, #2, #6, and #9, in the direction of the major difference displayed by trap #3, but not enough to be of concern in practical terms for the first year. If this difference is repeated in the second and third years, then it is an identifiable margin pertur-
bation effect that may be small at one point but if associated with many kilometers of road edge through primary forest, should be considered. The single-lane road itself eliminates a small portion of habitat near trap #7. This emphasizes the different kind of perturbation (even if shaded) that this trap received in the first year, in contrast to the other two margin traps (#3, #4).

Trap #3 is unambiguously very different from all the others both in numbers of specimens and the numbers of BINs (Figs. 5, 17–18, 20–21). The question becomes, what is the origin of the 5220 BINS not captured by any of the shaded forest traps? The open bare drilling platform is certainly not a living ecosystem that generates them. Traps #4 and #7 are viewed here as forest traps rather than edge traps, given the composition of their contents and the explanation to follow. The great increase in numbers of individuals and BINs in trap #3 is largely contributed by small parasitic Hymenoptera (Platygastridae, Ichneumonidae, Braconidae, Bethylidae) and parasitic and scavenging Diptera (Tachinidae, Phoridae). Quite surprisingly, the proportional increase is not made up of Cecidomyiidae gall-making and fungus-eating larval flies (Fig. 14). Cecidomyiids are the numerically dominant family taxa in all PL12 Malaise traps (and all ACG dry forest and rain forest traps as well). In other words, the proportionally most abundant family of insects did not disproportionately increase in the platform edge. This is probably because the true ecological origin of the Cecidomyiidae flying adults is largely in the forest understory, rather than the canopy or surrounding strongly insolated agroscape. This aspect will be explored in later years when the species composition by Cecidomyiidae in each trap has been more characterized taxonomically. It may also be associated with cecidomyiids being nearly all nocturnal and therefore less affected by 24 h Malaise trap positions relative to sun and shade.

It is highly unlikely that the parasitic and scavenging insects are greatly increased in the platform margin because of a hyper-abundance of food attracting them. In this first year, breeding populations of potential hosts have not had the chance to build up around the margins of the site (if they ever will). This process is especially the case in the first year of perturbation, when the heliophile vegetation of the platform margin is barely developed following the platform clearing and exposing of the forest understory (which was formerly in deep shade and therefore very poor in fast-growing leafy vegetation as food for insects, in contrast to the open insolated agroscape and forest canopy).

There are at least two potential non-exclusive sources for the small sun-loving insects that were so abundantly caught by trap #3. One source is that these are species that normally inhabit the forest canopy with its sunlight, wind, dryness, and heat. When the drilling platform is put into the forest, it introduces that microclimate downward to ground level, and these heliophile insects follow, continuing to search in or just above it, just as they do in the canopy.

The other source is, as mentioned above, the strongly exposed and insolated agroscape adjacent to the Pailas Dos forest. These old fields and pastures are variously beginning to regenerate young second growth forest that generates a steady rain of highly mobile (small) insects onto its surroundings (as waifs, strays, migrants,
and population explosions). When this insect rain falls on the intact forest canopy, it is largely if not entirely eaten by predators and scavengers and does not establish (due to lack of sunny microclimate and hosts). However, if a large insolated hole, such as the drilling platform, is created in the forest, then these flying insects will be attracted to the disturbance traits of the hole. It becomes an attractive microecosystem for them, irrespective of whether their usual prey is there. In the first year of the platform’s existence, conditions for their survival or population establishment are largely absent but we anticipate that as the margin of young secondary successional vegetation builds up around the margin of the platform, they may find progressively better circumstances for establishment and persistence at the PL12 drilling platform edge.

A final source of future change in the forest understory needs to be added to this complexity. Many sampling schemes in the ACG dry forest have shown that during the long dry season (January–May), many species and individuals of insects of secondary succession take refuge as sexually dormant adults in the cool, moist, and shady understory of nearby evergreen or semi-evergreen old-growth forest. This greatly, but just seasonally, increases the species richness in the shady microhabitat. When the rains come in May and there is leaf flush and shoot growth in the insolated (and now moist) secondary succession, these insects abandon the shady (and slow-growing) forest understory and move back into secondary succession (Janzen 1976). This phenomenon is expected in the forest of Pailas II, both near PL12 and near the other roads and drilling platforms. However, whether it has already begun in the first year of perturbation cannot be determined until the natural histories of the species in both forest and adjacent secondary succession are known and followed over subsequent years of sampling.

Compare traps #1, #2, and #3 as a potential gradient from disturbed to undisturbed forest understory, and likely future annual baseline biomonitoring for PL12

As mentioned earlier, it was anticipated that all nine Malaise traps would be analyzed. However, in 2016, funding was available to do only the three deep forest traps (#1, #6, #9) and the three traps at the edge of the perturbation (#3, #4, #7). When the opportunity arose to do one
more trap, #2 was chosen out of the three that were available at a distance of 50 m into the forest. This choice was because it is part of the logical biomonitoring line that is likely to continue into the indefinite future. This logical is because the 4-5-6 line was lightly perturbed by ICE expanding the platform in early 2016, and the entirety of the 7-8-9 line may have been impacted by ICE building a 30 m wide road parallel to trap #9’s forest at about 30–50 m distance.

In all respects, the contents of trap #2 are the same as the other deep shade traps, at the level of Ordinal, Family, and BIN counts, for this first year, and per week. Despite the drilling platform being conspicuously visible through the forest understory from trap #2, its captured material is essentially the same as that for traps #1, #4, #6, #7, and #9. Strikingly, its species accumulation curve is essentially identical to that of all the other shaded traps and dramatically different from that of trap #3 (Fig. 21). It differs only in that its total number of specimens is at the lower end of the range captured by each of all the other forest understory traps. While it is a sample size of one (to be replicated with future years by analysis of traps #5 and #8 with later funding), this result conforms strongly with the expected. As close as 50 m to the platform, the trappable insect community does not evidence disturbance for the first year of geothermal project activity.

Compare results derived from different intervals of trapping

It is easy to conjecture that more is better when it comes to how often to analyze Malaise trap samples. However, the weekly analysis gives the highest resolution for the purposes of biomonitoring. Whenever the samples were pooled into longer intervals, the resolution was reduced. When only every second week was sampled, or every fourth week was sampled there is a serious loss of resolution (not documented here). However, essentially all sensitivity to the dramatic changes with the rainy season can disappear if the wrong week is chosen. The same qualifier applies to the number of specimens per week. If we only count the number of BINs per month, pooling intervals appears to not matter, but there is very low resolution.

Fig. 20. Graph of BINs per week for all seven analyzed traps, along with rainfall and temperature. Trap #3, as discussed, is outstandingly different even though it conforms to the same general pattern through the year of the other six traps. Trap #2, even though being only 50 m into the forest behind trap #3, is indistinguishable from the other three deep forest traps, the platform edge trap (#4) with its collection bottle into the forest, and the road edge trap (#7) that is likewise pointed into the forest and also in the shade of the canopy left intact by ICE over the one-lane road. [Image: CBG coauthors, raw data from study.]
It is also relevant to consider the question as to whether the ecosystem around PL12 can be accurately portrayed through its insect composition by simply recording the number of specimens or their biomass rather than the number of BINs captured by the Malaise traps. As a beginning exploration of this possibility, Fig. 22 compares the numbers of BINs and of specimens in trap #3, the most specimen-rich of all traps. Both graphs (Fig. 22) show the same general pattern, but since there is no taxonomic information included, there is no detail in the baseline against which to compare future years.

Conclusions

This study is the deliberate melding of incomplete standard and innovative hard science with incomplete industrial necessity of rapid conservation and mitigation on a tropical frontier where massive amounts of wild biodiversity still exist but social desires for forest harvest or elimination are very active and expanding.

This year (2013–2014) of biomonitoring site PL12 of the Pailas II Geothermal Project conducted being developed by ICE was part of the 2016–2017 report to JICA as “Study on improvement of Environmental Monitoring methodologies for geothermal development in Costa Rica” (a.k.a. SAPI). The current environmental methodologies used by ICE and Costa Rica’s environmental monitoring institution, SETENA of MINAE, are well established, required for ICE and MINAE, and not under analysis here. Rather, the intent of this project by GDFCF/ACG, as part of the SAPI conducted through ERM (Japan) and funded by JICA, GDFCF, and ACG, was to begin to develop a new tool for ICE’s and JICA’s environmental biomonitoring tool kit.

Primary conclusions from the first year

1. A highly diverse tropical insect community, constituted of tens of thousands of undescribed species, can be used as a simple biomonitoring system to detect changes in biodiversity and abundance that are correlated with the perturbation by the geothermal project, and therefore be an index to the amount and location of that perturbation to biodiversity in general, since insects constitute at least 90% of the animal species-level Eukaryota biodiversity of a tropical forest.

2. Strategically placed Malaise traps capture thousands of species of flying insects per week, and therefore
can monitor short-term and long-term ecological responses to biodiversity perturbation; DNA barcoding and its analysis backed by voucher specimens now allows quick, cheap, and accurate identification of the trapped specimens, rather than requiring highly specialized and expensive labor-intensive months-to-years of professional taxonomic analysis (which can emerge later as desired). The process does not require extensive classical identification of the species being tracked. However, as usual, the more finely particulate the data, the more can be built out from it, both for project and generically. Simultaneously, the actual barcodes and their voucher specimens now become a taxonomically tractable barcode reference library and dictionary for all the many other scientific uses society has for an understood biodiversity (Janzen and Hallwachs 2019a).

3. Engineers and other geothermal project personnel with no formal biological training can accommodate and understand the required biological aspects of this new kind of biomonitoring, one that is conducted through laboratory-based DNA barcoding of the trapped insect specimens; this renders Malaise traps and what they capture into a technically intelligible methodology, such as engineers and other non-biologist sectors of society are comfortable with applying and learning from.

4. A geothermal project can understand and incorporate biomonitoring with Malaise traps coupled with DNA barcoding analysis as a useful technical component of an industrial project, just as are roads, bridges, drilling, platform sites, personnel, and administration, as well as are the classical kinds of short-term pre-project environmental monitoring (EIA) biomonitoring for endangered species or habitats.

5. It is preferable to collect baseline Malaise trap data for at least one year before any industrial work. This year of effort is also needed for standard EIA pre-industrial biomonitoring for endangered species or microhabitats; a year is required so as to encompass the normal seasonal fluctuations in community structure and species presence. However, if a year advance biomonitoring is impossible, results from somewhat distant Malaise traps in the same ecosystem can be proxies for a normal year baseline during the industrial work. Furthermore, these proxies have suffered the same weather as the biomonitors of actual or potential perturbation, thereby avoiding...

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**Fig. 22.** Comparison of trap #3, the one with the most BINs and most specimens, along with its cumulative monthly totals. At the level of crude BIN abundance and specimen abundance, mirroring each other and the weather changes (as shown in previous graphs), these results provide maximally simplistic baseline information for each of these traps. The blue bar is the period of drilling activity on the platform. They do suggest several directions for simple biomass and simple eDNA mass analysis, especially once the barcode library is more fully stocked. [Image: CBG coauthors, raw data from study.]
the problem of a pre-project year being seriously different weather-wise from the year of project initiation.

6. Engineers, planners, and other geothermal project personnel can fruitfully work with ecologists and conservation biologists to minimize the impact of the truly necessary aspects of a geothermal site (e.g., lights (Owens et al. 2020), forest clearing, people presence, earth moving, and noise). Thorough biomonitoring of all kinds may show that some of the anticipated perturbations are more contra-aesthetics and impression-based than actually damaging to the site biodiversity and its other ecological traits.

7. In this particular Costa Rican project, GDFCF (http://www.gdfcf.org) as a conservation NGO, plus its government partner, ACG (http://www.acguanacaste.ac.cr), can work calmly and constructively with the government geothermal company (ICE: https://www.grupoice.com) to create a new kind of long-term biomonitoring process for a geothermal drilling platform, and its access road, in sensitive and conservable old-growth tropical forest. It therefore prepares Costa Rica for consideration of a future within-park situation, inside the adjacent Área de Conservación Guanacaste UNESCO World Heritage Site, if it should ever be legally imposed.

8. Two quite different government agency subcultures (ICE and ACG) and a conservation NGO (GDFCF) collaborated intimately on this complex long-term project even though it required some conspicuous and innovative adjustments to some of the standard bureaucratic traits of these three subcultures, and some site-specific solutions to generic issues. It also required JICA as the fiscal patron for the entire biomonitoring project.

9. During the first year of biomonitoring with Malaise traps of site PL12 at different distances and orientations from the drilling platform, and the three months of drilling itself, there was no detected perturbation of the insect community, except for the actual clearing and its margins. During this first year, the geothermal platform perturbation is ecologically analogous to a 1.5 ha landslide, with respect to the overall biodiversity of the surrounding forest. While a landslide is eventually reoccupied by the adjacent forest, this will not be the case for the access road and platform. Their permanence is most analogous to a river edge within its adjacent forest.

10. In view of these results, a key question emerged: How many “landslides” or “riverbanks” of what size and configuration, and at what distance from each other, with what access roads, would be acceptable in a sensitive and still conservable old-growth forest that its society has major reasons to enter in a controlled manner? The answer varies from forest to forest and will need to be determined by on-site biomonitoring. The use of the same biomonitoring and analysis to track and measure the impact of climate change on highly heterogeneous tropical landscapes also requires serious feedback-rich and site-specific biomonitoring.

All of these 10 project goals were attained for this SAPI project in 2016 and early 2017, for the data from late 2013 to late 2014. They all show observational evidence of continuing to be the case for the PL12 project and other nearby ICE anticipated projects. They are both being used by GDFCF/ACG/SINAC, ICE, and JICA as examples of a positive interaction between conservation and industrial society.

In the process, these 10 goals have created the site-and project-specific result that, for this first year of biomonitoring, the drilling platform and its drilling activity, and its access road, have no visible perturbation effects on forest as close as 50 m distant from the platform edge. However, the platform does remove its biodiversity (except that which flies across it) and has a dramatic effect on its immediate border. Equally, observations by ICE and by GDFCF indicate that various vertebrates treat the PL12 site as a different, though not necessarily negative, microecosystem. There is no evidence that the presence of PL12 has a currently discernable effect on the great bulk of the biodiversity of Sector Pailas of ACG, Parque Nacional Rincon de la Vieja, and the ACG UNESCO Natural World Heritage Site as a whole.

The platform itself, an anthropological analog to a landslide or riverbank that eliminates about 1.5 ha of forest at a particular point, has created a very distinctive microperturbation in the form of a 3–10 m wide border that shares many biological and physical traits with the nearby old-fashioned agroscape and young secondary vegetation (as well as with the forest canopy itself). This narrow strip of heliophile vegetation and its biota is not to be confused or analogized with the sterilized and pesticided modern agroscape of industrialized rice, sugarcane, soya, pasture, or pineapple fields. On the one hand, there is the possibility that this old-time agriculturalization of the PL12 site will spread into the forest, thereby increasing the biological size of the perturbation over time. The ongoing analysis of the current six years of biomonitoring of PL12 of the frozen samples of the nine Malaise traps will document an ongoing presence or absence of such a biological spread. The specimens only lack barcoding budget to move forward with their analysis.

On the other hand, an understanding of the biology and biodiversity of this agriculturalized platform margin suggests possible design and treatments of it so as to minimize the possibility of its impact spreading. Any such modification would best be accompanied by further study of the biodiversity dynamics of tropical complex edge ecosystems, recognizing that each site will be dif-
ferent biologically as well as sociologically—one shirt definitely does not suit all. Ironically, landslides are an omnipresent habitat type in tropical species-rich forests on slopes (as are their flatland and sloping analogues—riverbanks). However, as a microhabitat type themselves, their insect biodiversity has received essentially no attention from tropical field ecologists (but see Krishnadas and Comita 2019 for a lead into plants on edges). A study (Janzen 1983) of a small area of primary old-growth dry forest in Sector Santa Rosa of ACG discovered that birds and other vertebrate seed dispersers move heliophile plants of adjacent secondary succession in abandoned agroscape into natural clearings from tree falls and forest understory. This gradually decomposes old-growth forest into a mix that is neither classic old-growth (with its disturbance by tree falls) nor pure secondary succession following catastrophic deforestation. This provoked the conclusion that for its preservation, a valuable old-growth primary forest might best be bordered by rice fields or clean pasture rather than the secondary succession, rich in vertebrates, much approved by conservationists as a buffer zone. The concept of a buffer zone is very deceptive; in human terms on a map it can look nice and reasonable, but in terms of wildland ecology versus human sociology, it may damage an adjacent old-growth forest more than help it.

Equally, it would be possible to collaborate with geothermal engineers to determine which aspects of standard drilling platforms and access roads could be modified to more exactly mimic the edge of a natural landslide or riverbank and thereby minimize the industrial impact on the forest in which the project occurs. This could involve the detailed configuration and placement of roads and platforms, the timing during the year of doing the clearing and drilling, the absence of lights (or the kinds of lights), and of course the exclusion of hunters or colonists and other unauthorized invaders from adjacent humanity, a highly destructive force that commonly makes use of forest penetration by roads, frontier colonists, and politicians.

There are two major considerations to viewing a geothermal industrial site (and its access road) as a species of landslide (or riverbank). First, it is a well-known concept to ecologists and other observers of nature, that light point-source disturbance to a large expanse of truly intact forest increases many aspects of its biodiversity on many axes. No sheep, species-poor pasture; many sheep, species-poor pasture; few to moderate sheep, species-rich pasture. In other words, how many landslides of what size and duration, and at what distance from each other, can occur in a large conserved forest without negatively impacting its overall biodiversity? The SAPI project focused on PL12 was not designed to directly answer this question, but its results, made possibly only by the CBG DNA barcoding, are relevant. In the practical terms of this particular geothermal site, one, two, or even three replicates of PL12, and its access road, carefully sited within 10 000–20 000 ha of forest of the kind hosting PL12 (note uniformity of forest appearance in Fig. 5), could well show no evidence of being different from several small landslides or a river of similar size. One to three one-time occasional landslides of 1–2 ha size in 10 000 ha of ACG old-growth forest will unambiguously increase that forest’s net biodiversity.

Second, there is another biodiversity consideration besides a simple accounting of the number of species and their interactions (a classical measure of biodiversity) as potentially being impacted by one to three geothermal drilling sites. An intact old-growth forest with no roads or other anthropological perturbations is an ever-rarer object on the globe. It is itself a threatened biodiverse object. Just as the human body can of course function equally well (or at times, better) with small and carefully placed surgeon’s scars or well-healed bullet wounds, there are parts of the body where even a very small and well-healed surface rupture lowers that human’s performance and survival. Location and intensity matter. In the particular case of PL12, the geothermal site and access road were chosen by ICE for their geothermal and engineering considerations before GDFCF/ACG biologists had the chance to collaborate. However, in a fully biodiversity-friendly planning exercise for an industrial geothermal site, the biodiversity traits of the proposed area of perturbation would be well understood biologically and the actual drilling sites and access roads positioned accordingly. Some sites would be simply unavailable because of what they contain or how they influence other sites. It is worth emphasizing that true biodiversity monitoring is concerned with both the immediate impact on focal species and microhabitats, and on the long-term impact on the overall biodiversity interactions of a conserved wildland.

All technical decisions need to consider the social bias against certain kinds of actions, quite irrespective of their measurable impact on the site’s biodiversity. It is viewed as quite acceptable to severely perturb 1–2 ha of old-growth forest for ecotourist accommodation and access, education, administrative/proective actions, or research. The same degree, and even kind, of perturbation is viewed with distaste or termed illegal when extracting a geothermal resource, mining, water capture, classical crops, or other quite socially visible commercial purposes. However, all perturbation actions are commercial ventures at some level, but some are declared legally legitimate in a national park and others as illegal. Some are carefully planned so as to not disturb the biodiversity and ecosystem values of the area, and many others are not. A road that subsequently gives access to colonists and hunters into a conservation area is a major threat, while a road with highly controlled use, such as that to PL12 or those throughout ACG, may be hardly more than analogs to small dry riverbeds. A road that transects a
unique, and today even-scarcer, old-growth ecosystem can be a travesty, while the same road in secondary succession on an abandoned pasture can be inoffensive and can be a travesty, while the same road in secondary succession on an abandoned pasture can be inoffensive and give legitimate management and approved access, along with society-wide desire and tolerable footprints.

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This report is derived from the much longer formal report to ICE and JICA entitled “Final (one year) report to ERMI/JICA/ICE from GDFCF with respect to BIO/CBG/Canada laboratory barcoding of PL12 Malaise samples” and then their subsequent analyses by GDFCF for the project entitled “SAPI for Geothermal Development Loan: Study on improvement of Environmental Monitoring methodologies for geothermal development in Costa Rica Agreement #0328497.” This longer report and copies of research, collecting, and DNA barcoding permits from the Costa Rican government are available on request to djanzen@sas.upenn.edu.

Several hundred different people with very different roles, many of which policy circumstances do not allow documentation here, have contributed to the ideas, execution, and writing of this report. We elect to briefly acknowledge the key players below as well as by their being classical coauthors. While this results in some persons being both a coauthor and acknowledged in the classical sense, this is appropriate given the very diverse, and sometimes unmentionable, roles each person has played. Melding two very different fields of social effort operating in four very different societies and countries requires unorthodox pathways.

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