



HOW A TROPICAL COUNTRY CAN DNA BARCODE ITSELF

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IBOL's new research program [BIOSCAN](#) has set out to DNA barcode the world (terrestrial invertebrates especially). This will give us a very broad and paper-thin view. We and the world can do a lot with paper. A very lot. Especially when it is used to map movements, detect presence, and begin to reveal the linkages among living things, tens of millions of living things.

Costa Rica, a very enthusiastic node in BIOSCAN, has decided instead to go for depth for a place, for its million-plus species of Eukaryota in an area the size of a tiny American state or a fraction of a Canadian province. There are more eukaryote species within 50 km of our field home in the Area Administrativa of Área de Conservación Guanacaste (ACG) in north-western Costa Rica^{1,2,3} than in all of Europe. Costa Rica's newly started ten-year goal is called BioAlfa, as

derived from BioAlfabetizada and BioAlfabetización (a.k.a. BioLiterate in another familiar language).

BioAlfa aims to document and reveal, for its five million people, by those people, with appropriate international collaborations and one major technical advance, the pocket personal reusable cheap barcoder, Costa Rica's "total multi-cellular biodiversity". For any species, if you know what it is, if you can read and access it, then humans are quite adroit at deciding how and why they want to conserve it for truly sustainable uses. "Use it or lose it" is a gene complex deeply embedded in our Pleistocene genome. That is the deepest, most selfish driver of BioAlfa. A thoroughly barcoded national biodiversity will be at the least a major reference baseline for whatever sampling methods BIOSCAN and others employ world-wide. Not only will BioAlfa have repeatedly collected a year of

Malaise traps from this or that ecosystem, but it will also come to know what fraction of the trappable and untrappable arthropod fauna is being captured in that or other years, owing to both Malaise trapping and all the other many ways we find invertebrates.

For any organism found in the country, Costa Rica wants to know what it is, where it is, what it does, and how to find it when you want to – for at the least the 25% of the country that is still natural forest – dry forest, rainforest, and (rapidly dwindling) cloud forest. And what is on the agroscape derives from the wild as well, so it will be registered too. BioAlfa’s technical and bio-political goal is to capture those informative items and get them all readably and legally stashed on the internet in the public domain. Until the internet, computerization, and DNA barcoding emerged, such a goal would have been only a dream, and restricted largely to what is big enough for you see through your binoculars or in the museum drawer. The latter species are some 2% of Costa Rica’s wild biodiversity, depending on how good and trained your vision, and how many libraries and field guides you have to hand – along with ever-scarcer quality taxonomists. What about the rest of the crops, beauties, wiggles, and dangers in those wild forests and remaining freshwater – 70% arthropods, 19% nematodes, and 9% fungi?

BioAlfa is modular. Different modules range from taxon-based morphology+barcoding inventory (e.g., all Hymenoptera, all Lepidoptera) that was initiated decades ago^{1,4,7}, to industrial biomonitoring⁸ with six years (so far) of Malaise traps to determine the impact of a road and geothermal drilling platform on original complex tropical forest, to facilitating staff of Costa Rica’s national parks to set and manage at least a year of Malaise traps in all of Costa Rica’s major ecosystems (including the urban capital San Jose) with their own sweat equity (initiated March 2019 by gov-NGO collaboration), to (taking a note from iBOL’s Canadian efforts) setting rural schools to do their own [Malaise trapping](#). And in all of this, BioAlfa is turning Costa Rica into being a tropical canary in the coal mine, now facing three decades of climate change⁹.

As BioAlfa takes form, perhaps being a walking organism by January 2020, the modules each move at their own pace according to their technology, biopolitics, and “small” funds to accompany their massive Costa Rican (and on many occasions international) sweat equity. The sweat-equity budget will always characterize sustainable long-term tropical national

biodiversity biodevelopment but also is the only way known to have a tropical country itself become truly aware of this vital piece of its socioeconomic fabric. The modules also fit together according to their development, their discoveries, their small failures, their repairable flat tires. This is planning by doing rather than hewing to a monster complex “plan” or “blueprint” that is long obsolete by the time the rubber hits the road and takes years to create in the first place. It is also evolution, as biologists are very aware. But for other sectors of society, this methodology can be quite novel and even threatening.



BioAlfa Module: Morphology+Barcoding Inventory
Hazel Cambrero and Sergio Salas collecting ACG moths from a light trap. Each moth is individually collected into discardable plastic containers so as to minimize DNA contamination among them. At present, 11,500+ species of ACG Lepidoptera have been barcoded from light-trapped adults since 2004 and reared caterpillars since 1978, for an estimated total of 15,000 species from an area the size of New York and its suburbs (125,000 terrestrial ha of dry forest, rainforest, cloud forest, and intergrades of a 400-year old ranch being restored since 1985).

There is, however, the reality that the overall cash cost, to match at least a portion of Costa Rica’s sweat equity (coupled with the same from the international community, and especially the taxisphere) will be at least \$100 million as a single source spread over ten years and variously front-loaded and pulsed. Of this, \$25 million will be needed for a permanent endowment, the income from which will be aimed at perpetuity for all of BioAlfa’s processes – ranging from data and specimen preservation to constant updating of information management for raw information and output formats. Another \$25 million will be a slowly sinking fund to facilitate start-ups and start-up processes that build on BioAlfa biodiversity data for



BioAlfa Module: Industrial Biomonitoring

The PL12 geothermal drilling platform in its first year of construction, with a Malaise trap at its margin for biomonitoring (the white spot just to the left of the yellow star). This trap and its eight companions have been in place since forest clearing began in September 2013. Together these traps continually compare the insect community of the margin with that 50 m and 150 m into the forest behind. This trap collected ~ 80,000 insects of ~8,500 species in its first year, the same year that the platform was carved out of this old-growth forest. Comparison with the traps 50 and 150 m into the forest showed that as far as their species and seasonal communities were concerned, the drilling platform did not exist. These nine traps in total contributed 11,500+ species barcodes as measured through Barcode Index Numbers. The actual number once these species are “more known” will be about 13,000. The Centre for Biodiversity Genomics did the barcoding and analyses of seven of the traps in six months with funding from the Japan International Cooperation Agency (JICA) (the other two traps are still in the freezer, awaiting funding for processing).

biodevelopment of all kinds by all sectors – government, private, commercial, academic, etc.; innovative embedment of wild biodiversity in Costa Rica’s socioeconomic fabric is the goal. And, finally, \$50 million is for the actual cost associated with all the mechanics of species sampling (no matter how much sweat equity, there are store-bought costs), laboratory DNA barcoding the samples, and base management of the information and process. Fortunately, BioAlfa has the Centre for Biodiversity Genomics (CBG) backing BIOSCAN. The CBG can also meet the BioAlfa need to DNA barcode at least 10 million specimens that will be generated by BioAlfa over its ten years; the CBG has already barcoded 500,000+ ACG insects of about 45,000 species since ACG offered itself in 2003 to the CBG for this close scrutiny of tropical biodiversity, a level of scrutiny of which we have been innocently ignorant for various centuries.

A magnificent DNA barcode library and its accompanying collateral data and analyses will not, however, truly generate national bioliteracy until every citizen – farmer, schoolchild, housewife, garage mechanic, entrepreneur, and government employee has or can have a dirt-cheap personal barcoder in their pocket, reusable and connected to the internet through Wifi. All of the quasi-analogues that exist today and those envisioned are aimed at the high-end purchaser of both gadgets and supplies. When a cheap alternative comes down the road, there will be billions of buyers and barcodes and collateral information on each species will be the resource in short supply. Until that time, both verified barcodes and the gadget are in short supply. A bioliterate world will contribute seriously to the global barcode library every time anyone uses the magic gadget. As in literacy, for it to reach its social potential, cheap tools are needed for everyone.



BioAlfa Module: Facilitating National Park Staff’s Efforts

Two staff of Parque Nacional Tortuguero install their first Malaise trap by themselves following their early March instructive workshop in SINAC’s Parque Ecologico in Santo Domingo de Heredia (outskirts of San Jose). While the photo appears simply to show two researchers, closer scrutiny shows that they both have guns on their belts. That is to say, they are simultaneously park guards on the government payroll while now performing biodiversity science. The trap is about 150 m from the jaguar at the bottom, from a video taken by the Costa Rican Minister of the Environment himself, Carlos Manuel Rodriguez, on a visit at about the same time. The slide itself was created by the trap setters and posted by them, unknown to BioAlfa, on International Forest Day, 21 Mar 2019.

As is known to all, the academic research and discovery community has for a very long time been examining portions of tropical biodiversity for a multitude of reasons, yet also mostly in the fragmented, expeditionary and superficial way common to our

northern societies and their tropical acolytes. BioAlfa is philosophically and technically an offspring of those efforts, but in modular outcomes differs in audience and actions. Here we list just a few as modules of the start-up process. Each document is available on request for updates, if there are, but most are now available on the [ACG web site](#) and more will be parked there as available.

1 On 27 November 2017, the previous Costa Rican government decreed that DNA barcodes from Costa Rican specimens are public domain, and therefore may be harvested (under appropriate government permit from CONAGEBIO, the operational manifestation of Costa Rica's Ley de Biodiversidad¹¹). Costa Rican barcodes can be freely used, published, etc. for biodiversity identification and discovery. This assumes that the barcode itself is not of commercial value, anymore than is a word in a dictionary. Indeed, CONAGEBIO's headline is "Contributing to the conservation and sustainable uses of biodiversity". The permitting process basically involves registration of the project coupled with assurances that the actual owner of the organisms is in agreement with the sampling of "his or her" beasts. Simultaneously this decree set in motion the government mechanics of formally bringing BioAlfa to life. Available on request and the ACG web site (decree #40725).

2 On 17 November 2018, the staff of the Sistema Nacional de Areas de Conservación (SINAC) of the present Costa Rican government of President Carlos Andrés Alvarado Quesada presented BioAlfa to the global COP meeting of the CBD (Convention for Biological Diversity) in Egypt as part of Costa Rica's ongoing stock-taking of its biodiversity resources and weaving them into national socioeconomics. A bilingual folder available on request and the ACG web site.

3 On 4 June 2019, the Costa Rican government officialized BioAlfa and its goals, especially by declaring it to be a process of National Importance, thereby granting even more legitimacy for the government to develop government-NGO-private-commercial-academic collaborations to advance BioAlfa goals (decree #41767). The signing of this decree was the occasion of a sit-down explanatory meeting of BioAlfa with the entire Cabinet and other major sector decision-makers. It is also at the roots of the government of Costa Rica currently constructing a proposal to the government of Norway for major funding of BioAlfa (fingers crossed).

4 At present, a number of small modules are being initiated or continue with the few resources at hand, while desperately searching for at least enough funding to cover CBG's sequencing costs that are currently at \$3/insect for massive samples and hoped to drop to about \$1/insect by January 2020.

- Expansion of the long-on-going intense inventory of ACG Lepidoptera (adults and caterpillars and their parasitoids) to include the remainder of Costa Rica (estimate at least 25,000 species of Lepidoptera).
- DNA barcoding the thorough national collection of aquatic insects in ethanol, done a few years earlier by the Universidad de Costa Rica but with no funds for identification.
- Six years of continued barcoding and analysis of the 9 Malaise trap catches from the geothermal biomonitoring site and at select other National Electric Company (ICE) rural installations⁸.
- Setting and managing Malaise traps by park staff, collected weekly for at least a year in 9 national parks (24 traps).
- Malaise trapping conducted and analysis paid for by commercial ecotourism lodges and hotels in rural areas.
- Ongoing organizational meetings with the Ministry of Education for the enlistment of select rural highschoools to run Malaise traps in their vicinities.
- Initial conversations with farm owners and government facilitators for combining insect trapping in fields with adjacent wild vegetation.
- Initiation of conversations for collaborations with image, natural history and genomic aggregators such as GBIF, iNaturalist, and all the other mega-initiatives such as the Earth BioGenome Project (EBP). BioAlfa, just as is the case with BIOSCAN, has much to offer all of them.
- Initiation of the dedicated BioAlfa web site. This is currently parked on the ACG web site¹⁰, but that will eventually have its very own encyclopedic web site.
- Initiation of the process of quickly blessing enormous numbers of undescribed species with classical scientific names, and at the same time handling species-level taxonomic information flow and analysis with COI barcodes and

BINs. It is a commonplace in the Costa Rican species-rich ecosystems for a BIN to contain several species that can be discriminated both by other subtle (to a human) traits and by clean clustering on both sides of shallow barcode splits. The latter discrimination is greatly facilitated by large sample sizes accompanied by accumulating collateral information on natural history.

- Initiation of forming a technical-biopolitical team of seasoned biodiversity managers with basic office facilities in San Jose (actually, Santo Domingo de Heredia in the outskirts of San Jose) and in ACG. On 1 January 2019, the BioAlfa team was joined by Jenny (Eugenie) Phillips Rodriguez (phillipsrodriguez@gmail.com), the primary Costa Rican microlepidoptera specialist who has dedicated decades to the former IN-Bio collection (now legal property of the Museo Nacional de Costa Rica) and its antecedents and its parataxonomists, and MS Roberto Fernandez Ugalde (Robferug2000@gmail.com), formerly a field environmental planner for ICE projects, ecotourist guide and herpetological aficionado. They are supported by the GDFCF¹² endowment constructed for the specific purpose of supporting the long-term survival of ACG biodiversity, which of course includes its integral role in BioAlfa.
- And many more at various stages of seeding or growth, but all delayed by the lack of funds for the direct laboratory sequencing to build the Costa Rican DNA barcode public library in BOLD.

In short, BioAlfa is engaging in the technically straightforward task of DNA barcoding an entire tropical country so as to set it up for being sustainably wanted as wild organisms by its own country. This is simultaneously a massively complex collage of technical science embedded in an omnipresent biopolitical socioeconomic context. BioAlfa will only be achievable when actually carried out by the future owners and caretakers of their country, with support from appropriate international collaborations.

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