The Rufford Foundation
Final Report

Congratulations on the completion of your project that was supported by The Rufford Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	Sheila Rodríguez Machado
Project title	Assessing genetic diversity of the Cuban endemic fish *Limia vittata* (Poeciliidae): implications for its conservation
RSG reference	17653-1
Reporting period	July, 2015-July, 2016
Amount of grant	£5000
Your email address	sheilaroma89@gmail.com
Date of this report	August, 2016

1. Please indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
To analyse the phylogenetic and phylogeographical relationships and genetic diversity among *Limia vittata* populations in Cuba			x	
To identify cryptic lineages (hidden diversity) with conservation needs within *L. vittata*		x		- We confirmed *L. vittata* as a single taxonomic unit, *i.e.*, we did not find cryptic lineages within *L. virttata*, at least with the mitochondrial genes cytochrome oxidase I and control region. However, according to our census we did find an increasing number of populations with very small number of individuals (two to three), mostly in eastern Cuba and several anthropogenic impacts affecting them. - Additionally, we found evidence of translocations of *L. vittata* fishes.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

In general, our project was done according the schedule. The main inconvenience was the sample collection in some of the most contaminated rivers and streams where it was impossible to get into the water. When possible we sampled alternatively in another water body of the same basin. On the other hand, given that we did not use invasive methods as electrofishing that allows to collect more individuals *per* time unit, the sampling of populations with low number of individuals took us more time than expected.

3. Briefly describe the three most important outcomes of your project.

1) For the first time we could explore the molecular phylogeny of *Limia vittata* based on two mitochondrial genes (cytochrome oxidase I and the control region) of individuals belonging to 52 populations (more than 100 sites visited) across the Cuban archipelago (Appendix I). We found a high level of haplotype diversity and relatively low nucleotide diversity within this species. Moreover, we found evidence of *L. vittata* translocations using molecular tools. This was a typical practice in Cuba during the 1990s when fish were used for plague control (mostly mosquitoes) and/or for aquarium trade. We found at least two populations in eastern Cuba where fishes are more closely related to populations from western Cuba, and even there is a population in which some individuals have a western haplotype and others individuals the eastern haplotypes.

We found a phylogeographical pattern which was consistent with those found in other group of fish, frogs and reptiles in the island. This implies that geological historical events have shaped the evolution of vertebrates in Cuba.

2) We visited many sites that represented new locality records for several species of Cuban freshwater fish, not only for the genus *Limia* but also for others genus like *Gambusia* and *Girardinus* (Poeciliidae), *Agonostomus* (Mugilidae), *Anguilla* (Anguillidae) and *Alepidomus* (Atherinidae). Also, we recorded most of the threats faced by our freshwater ichthyofauna (Appendix II, 2nd poster). These can be summarised as follows: fragmentation and habitat lost, introduction of invasive exotic species, dumping of household and industrial waste and changes of natural watercourses.

3) The dissemination of our results was one of the most significant results of the project. We presented our findings in important international and national meetings (Appendix III). Additionally, we gave talks to conservation authorities and to local community people on the identification of Cuban species, on the anthropogenic actions negatively affecting them and on the conservation status of the endemic ones. For the first time in some protected areas and communities they knew about the Cuban ichthyofauna. In fact, in one of these areas we gave them the tools to elaborate the management plan of the freshwater fish species (Appendix IV).

4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

Despite sometimes people seemed indifferent at the early beginning of oral presentations, in all cases once we started to talk they were very interested and motivated with the topic and they asked many questions. They learned about our diverse freshwater fish fauna, the main threats affecting them and how they could have an active role on their management and conservation, especially by capturing invasive exotic species and not making translocations of native species without previous enquiry. Moreover, we printed posters and water-proof field guides that were distributed in key places both in local communities and protected areas (see Appendix II)

5. Are there any plans to continue this work?

With this project we had the opportunity to sample many places in Cuba and to get an overview on the threats faced by freshwater fish and their habitats. In this sense, the Central and Eastern regions of the island have been severally affected by hill farming and deforestation. Thus, they are key areas for further and more thorough studies.

On the other hand, we plan to continue working on the genus *Limia* by extending our research on the diversity of species living outside Cuba and this way on the identification of new conservation issues. It has been a neglected group of fish for more than a century. The conservation status and basic information of most species are completely missing.

Additionally, recent molecular researches have confirmed the existence of at least two new endemic species from the families Eleotridae and Synbranchidae in Cuba, the latter widely used as bait in freshwater fishing. Thus, this is a topic that would deserves extra conservation efforts, including the collection of some critical information to make the formal description of some of these species.

6. How do you plan to share the results of your work with others?

We already shared most part of our results through two meetings focused on biological conservation: the XIX Congress of the Mesoamerican Society for Biology and Conservation *SMBC* (Mexico) and the II Scientific Event of Biological Diversity *BiodiverSOS* (Cuba) (see Appendix III). Additionally, in all meetings with technical staff form protected areas and people form local communities, we discussed the importance and threats faced by freshwater fish, its diversity and how they could contribute to its conservation.

Our results will be also available through my master thesis at the library of the *Centro de Investigaciones Marinas de la Universidad de La Habana* in both digital and printed formats. Finally, we will publish the results in a peer reviewed journal next year.

As a collateral result of this RSG project, we are finishing a scientific article on the distribution range of another poeciliid species (*Girardinus falcatus*) thanks to the data gathered during our field samplings.

7. Timescale: Over what period was The Rufford Foundation grant used? How does this compare to the anticipated or actual length of the project?

We planned to start the project in June 2015. We used the grant between August 2015 and July 2016. The actual time schedule for field and laboratory works was as we planned, except for some last minute field trips at the early beginning of the project.

8. Budget: Please provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used.

Item	Budgeted Amount	Actual Amount	Difference	Comments
2 Nylon Minnow Seine	200	100	+100	We received one from donation
2 Aquatic net	130	70	+60	We received one from donation
2 Pairs of diving boots	80	40	+40	We received one from donation
2 Backpacks	240	200	+40	We found cheaper options
2 Tents	200	150	+50	Price varies in the market
2 Sleeping bag	100	100	0	
Laboratory suppliers	900	1190	-290	This difference was took it from the costs saved above
2 Vary-Case Dissecting kit	40	36	+4	
1 Laptop	400	390	+10	
1 GPS	150	130	+20	It was impossible to enter a GPS because laws at that moment and special permits in Cuba. Thus, we bought a Smartphone with GPS

				that was enough for our goals
Travel expenses	400	380	+20	
Fuel	700	600	+100	Fuel price decreased
Food	500	400	+100	Reallocated in producing educative material
Communications (phone/internet)	250	200	+50	
Shipping costs	500	400	+100	There were less products to buy and send
Contingencies	200	0	+200	Before I win the grant I proposed to reallocate the contingency budget for producing educative material
Education material	0	300	-300	Used from the Contingences and Food
Bank transfer (3%)	0	150	-150	Reallocated from Fuel and Communications
Total*	**4990**	**4836**	**+154**	

*Exchange rate (March, 2015): 1£ = 1.48 CUC (Cuban Convertibles Units) – Application submission; Exchange rate (June, 2015): 1£ = 1.51 CUC – Project approval; Exchange rate (October, 2015-July, 2016): 1£ = 1.40-1.28 CUC – Project implementation

9. Looking ahead, what do you feel are the important next steps?

Given the high level of pollution in many rivers, mostly in those crossing towns and cities, and the lack of knowledge on the diversity of the Cuban freshwater fishes it is important to keep sensitising people from local communities and enhancing capacity building of technical staff from protected areas, particularly from central and eastern Cuba, the least studied regions.

It will be also important to achieve the inclusion of freshwater fish in the study and monitoring plans in protected areas. Recently we analysed data from our National System of Protected Areas (SNAP), and we realised that freshwater fish are poorly represented in their management actions plans. In this sense, as a first approach we are writing a book chapter about techniques and protocols for freshwater fish monitoring and inventories, including the methods of collection and preservation. Finally, I will defend my MSc thesis by the end of this year and we will publish our results in an international peer reviewed journal next year.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the RSGF receive any publicity during the course of your work?

Our conservation efforts were focused on both scientific community and technical staff and people from local communities. For that we made two presentations in scientific meetings and we gave talks and produce posters and field guides in some of the visited areas in Cuba. In all cases we recognise the important contribution of the RSGF by using the logo. Likewise, we will acknowledge the RSG in my thesis document and in all further publications derived from this project.

11. Any other comments?

I am very grateful to Rufford Small Grant Foundation for allowing me to complete my master degree thesis and to start a conservation program for the study and conservation of Cuban freshwater fish in two of the least studied regions of the country, central and eastern Cuba. We also acknowledge the support provided by one of our referees, Dr Didier Casane, who made valuable contributions during the project.

Once finished my thesis dissertation and our results published, I will send a copy of both documents to the RSG board. I will also send a copy of the paper on range distribution as well as of other publications based on the field work of this project.

Final Report

Project 17653-1

Assessing genetic diversity of the Cuban endemic fish *Limia vittata* (Poeciliidae): implications for its conservation

Appendix I. Summary of field work

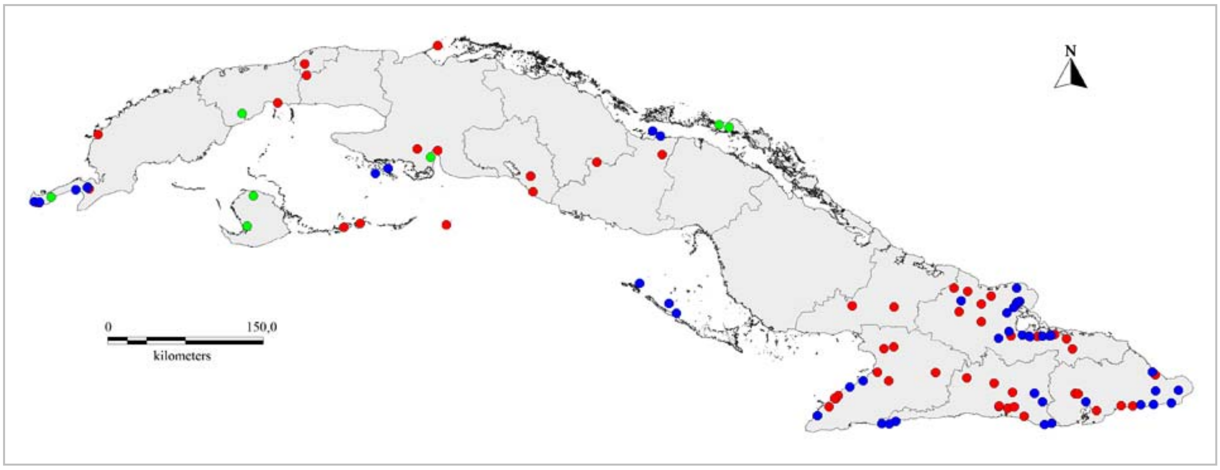

Figure 1. Map depicting the localities visited during the project across the Cuban archipelago. Red dots represent localities harbouring *Limia vittata* fishes. Green dots represent previous records

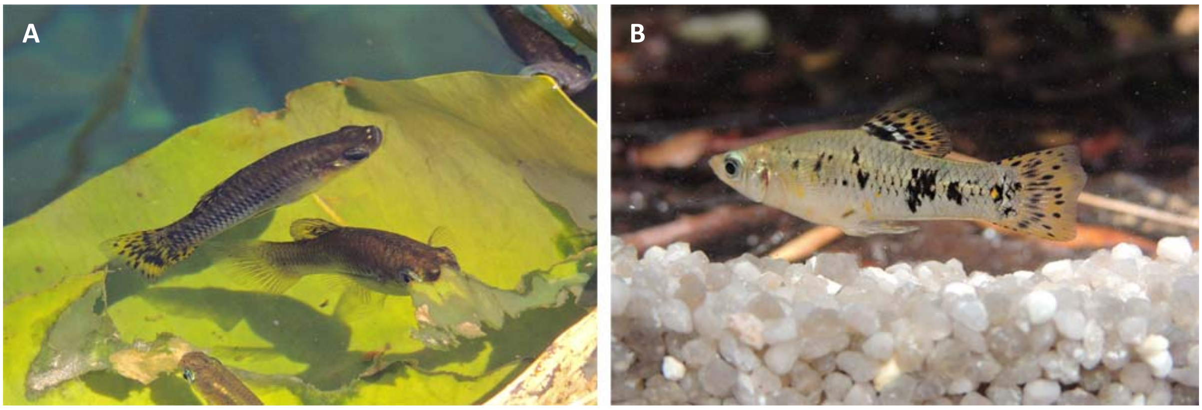

Figure 2. *Limia vittata* fishes in Jagüey Grande, Matanzas province (A) and in Guanímar, Artemisa province (B)

Figure 3. Typical habitats of *Limia vittata*. Jagüey Grande, Matanzas province (A) Cabrales stream, Villa Clara province (B), Cacoyugüín river, Holguín province (C) and Miel River, Guantánamo province (D).

Figure 4. Samplings of *Limia vittata*. Caleta Larga, Pinar del Río province (A), Mayajigua, Sancti Spiritus province (B), Cacoyugüín river, Holguín province (C) and Vicana river, Granma province (D).

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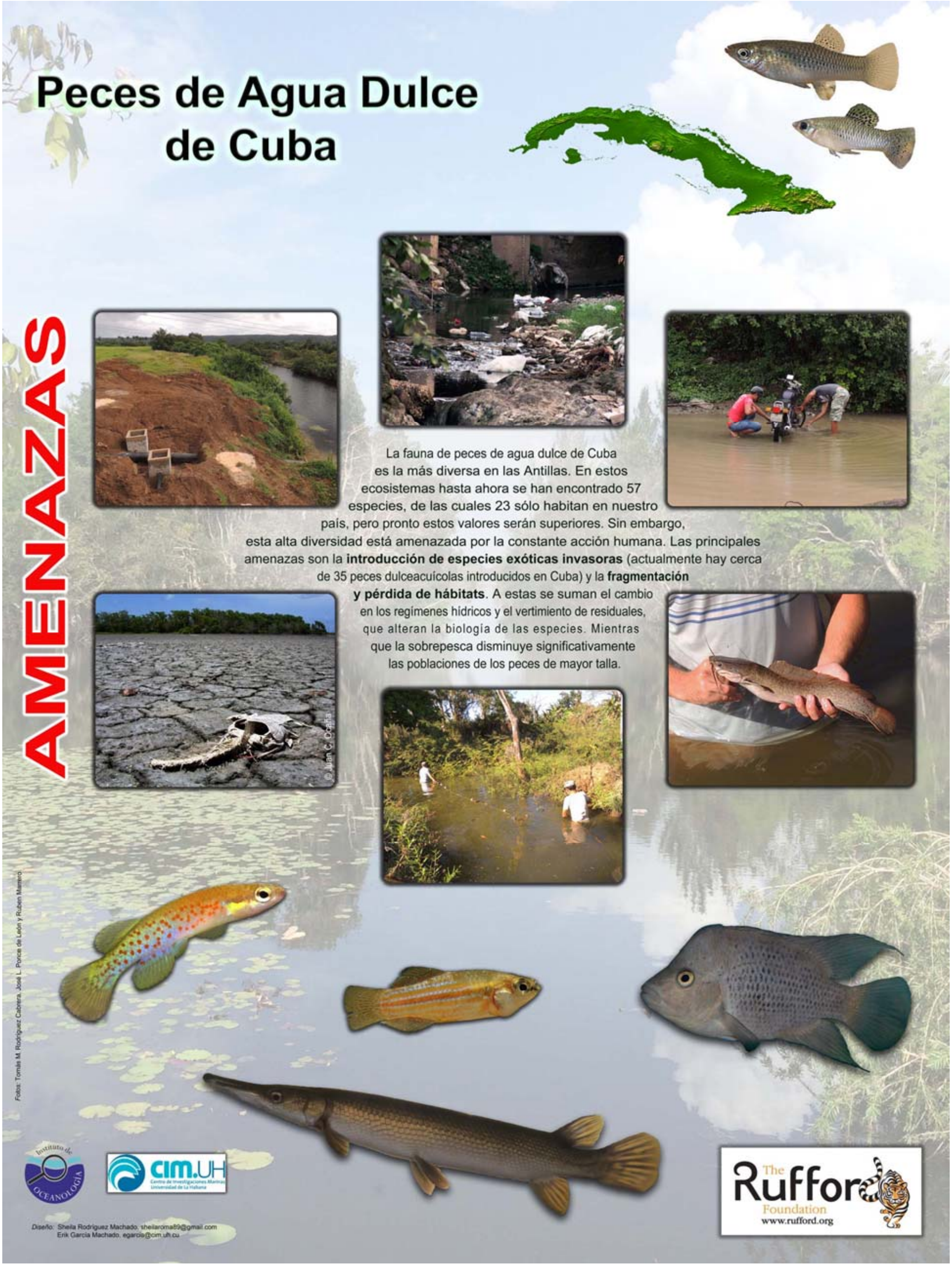
Appendix II. Educative material

Poster



Peces de Agua Dulce de Cuba
AMENAZAS
La fauna de peces de agua dulce de Cuba es la más diversa en las Antillas. En estos ecosistemas hasta ahora se han encontrado 57 especies, de las cuales 23 sólo habitan en nuestro país, pero pronto estos valores serán superiores. Sin embargo, esta alta diversidad está amenazada por la constante acción humana. Las principales amenazas son la introducción de especies exóticas invasoras (actualmente hay cerca de 35 peces dulceacuícolas introducidos en Cuba) y la fragmentación y pérdida de hábitats. A estas se suman el cambio en los regímenes hídricos y el vertimiento de residuales, que alteran la biología de las especies. Mientras que la sobrepesca disminuye significativamente las poblaciones de los peces de mayor talla.
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Fotos: Tomás M. Rodríguez Cabrera, José L. Ponce de León y Rubén Marrero
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Poster

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Poster

Water-proof field guide

Appendix III. Dissemination of results

Figure 1. Oral presentation (A) and closing and award ceremony gala (2nd Award!) in the Congress of the Mesoamerican Society for Biology and Conservation, México

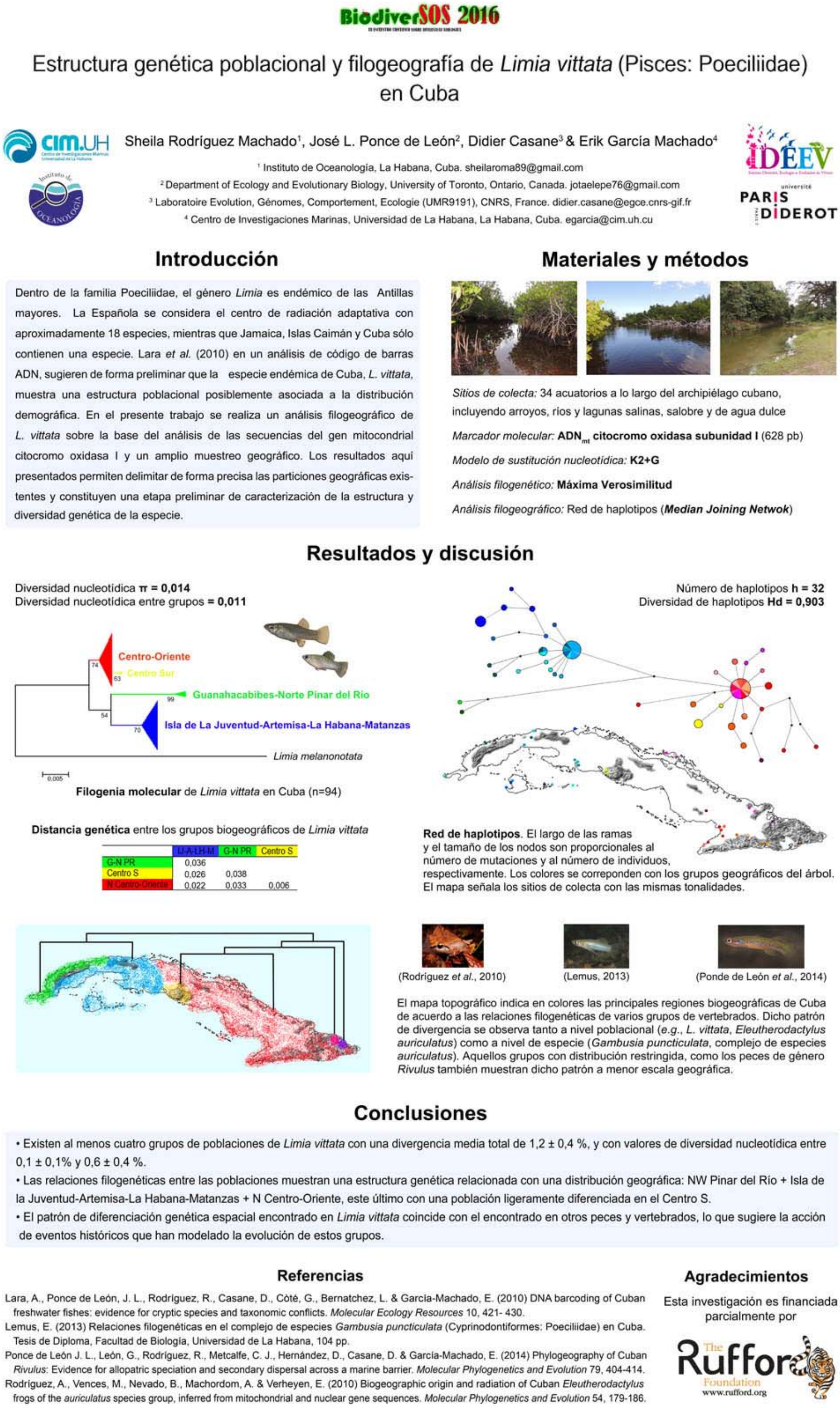
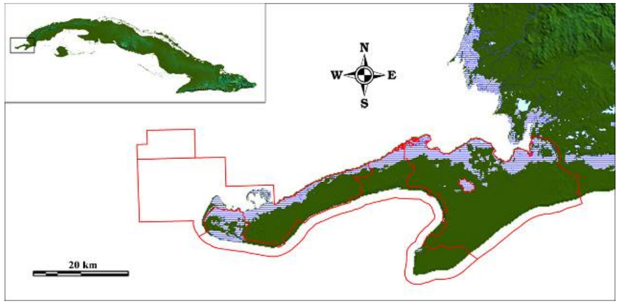


Figure 2. Poster presented in the II Scientific Event for Biological Diversity *BiodiverSOS*, Cuba

Figure 3. Faculty of Biology, Universidad Central de Las Villas, Villa Clara province (A), Sabanas de Santa Clara Floristic Reserve, Villa Clara province (B), La Bajada community, Pinar del Río province (C) and Castillo de Jagua community, Cienfuegos province (D).

Appendix IV. Field Report Península de Guanahacabibes Biosphere Reserve



Peces de Agua Dulce de la Reserva de la Biosfera Península de Guanahacabibes
Resultados preliminares. Viaje de campo 29 de febrero–1 de marzo, 2016

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Familia	Especie	Nombre común	Distribución y endemismo	Ecosistema	Grado de amenaza (IUCN/Libro Rojo de Cuba)	Imagen
Poeciliidae	*Gambusia punctata*	Guajacón	Cuba* (**E**)	Zonas de montaña y zonas bajas	No Evaluada	
	Gambusia puncticulata	Guajacón	Cuba*, Bahamas, Jamaica e Islas Caimán (**N**)	Zonas bajas	No Evaluada	
	Girardinus metallicus	Guajacón	Cuba (**E**)	Zonas bajas	No Evaluada	

	Girardinus falcatus	Guajacón	Cuba (**E**)	Zonas bajas	No Evaluada	
	Quintana atrizona	Guajacón	Isla de la Juventud. Doadrio *et al*. (2009) la reportaron para la Península de Guanahacabibes (**E**)	Zonas bajas	No Evaluada/En Peligro	
	Limia vittata	Guajacón	Cuba (**E**)	Zonas bajas	No Evaluada	
Cichlidae	*Nandopsis tetracanthus*	Biajaca Criolla	Cuba (**E**)	Zonas bajas	No Evaluada	
	Cyprinodon variegatus	-	Cuba, las Antillas, costa Este de Norteamérica, desde Massachusetts hasta el Norte de México, costa Norte de Sudamérica (**N**)	Zonas bajas	Preocupación menor/No Evaluada	

Cyprinodontidae	*Cubanichthys cubensis*	Neón cubano	Occidente y Centro de Cuba. Reportada para la Península de Guanahacabibes por Lee *et al*. (1983) (**E**).	Zonas costeras	No Evaluada	
Atherinidae	*Alepidomus evermanni*	-	Costa Sur de Cuba (**E**)	Zonas bajas, ecosistemas lénticos (lagunas)	No Evaluada	
Anguiliidae	*Anguilla rostrata*	Anguila	Cuba, costa Este de Norteamérica desde Canadá hasta Panamá, las Antillas hasta Trinidad (**N**)	Zonas bajas, acuatorios con flujo continuo de agua. Especie catádroma (vive en agua dulce y desova en el mar)	En Peligro/No Evaluada	
Mugilidae	*Agonostomus monticola*	Dajao	Cuba, las Antillas, costa Este de Norteamérica desde Carolina del Norte, Luisiana y Texas hasta Colombia y Venezuela (**N**)	Ríos de montaña, desembocadura de ríos y esteros	Preocupación menor/No Evaluada	

Bythitidae	*Lucifuga dentata*[Φ]	Pez ciego	Occidente de Cuba (García Machado y Hernández Martínez, 2012) (**E**)	Cuevas en la llanura meridional	Vulnerable/Casi amenazada	

***** Pendiente de revisión taxonómica y cambio de distribución luego de que se publique el estudio genético del género *Gambusia* en Cuba

[Φ] Pendiente de revisión taxonómica. Resultados genéticos muestran que esta especie comprende tres linajes independientes. En la Península de Guanahacabies deben quedarse dos especies, una de ellas endémica local de Guanahacabibes

E: Endémica
N: Nativa

Lagunas muestreadas y especies en cada una

Laguna	**Coordenadas (Lat., Long.)**	**Especies**
El Veral	21.93692°N, -84.53243°W	*Nandopsis tetracanthus**
Caleta Larga	21.83174°N, -84.91871°W	*Gambusia puncticulata, Ga. punctata**
Estero Alto del Negro	21.82603°N, -84.89411°W	*Limia vittata*, Ga. puncticulata, Ga. punctata*, Agonostomus monticola*
Caleta del Piojo	21.82482°N, -84.87059°W	*L. vittata*, Ga. puncticulata*
Los Conucos (El Cristal)	21.8294°N, -84.86894°W	*Gambusia* sp. (juveniles), *Cyprinodon variegatus*
San José	21.96062°N, -84.42135°W	*Ga. punctata*, Girardinus metallicus*, Gi. falcatus*, N. tetracanthus**
Las Coloradas	21.95605°N, -84.42339°W	*Ga. punctata*, Gi. metallicus**
Lugones	21.94426°N, -84.40769°W	*L. vittata*, Ga. puncticulata, Ga. punctata*, Gi. metallicus*, Gi. falcatus*, Alepidomus evermanni*, N. tetracanthus**

* Endémicas

Referencias

Doadrio, I., S. Perea, L. Alcaraz y N. Hernández. 2009. Molecular phylogeny and biogeography of the Cuban genus *Girardinus* Poey, 1854 and relationships within the tribe Girardinini (Actinopterygii, Poeciliidae). *Molecular Phylogenetics and Evolution* **50**, 16-30.

García Machado, E. y D.A. Hernández Martínez. 2012. *Lucifuga dentata dentata*. *En*: Libro Rojo de los Vertebrados de Cuba. H. González, L. Rodríguez-Schettino, C. Mancina e I. Ramos (eds.). Editorial Academia, La Habana. 37-39 pp.

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