### Isabelle M. Vea

Evolutionary biologist passionate about scale insects and other bugs



## Insects found on dinosaur feathers are not that kind of parasites

In December 2019, I was in New York City, and while waiting for my husband to recover at the hospital, I came across an article in The Guardian showing a photo of an insect in amber that looked very familiar to me. After reading the title: **Dinosaurs had feathers ruffled by parasites, study finds**. My first thought was : Very weird, this really looks like a baby scale insect. I should take a look at the original article later.

# Dinosaurs had feathers ruffled by parasites, study finds

## Ancient pieces of amber found to contain dinosaur feathers riddled with louse-like insects



A Mesophthirus angeli feeding on dinosaur feathers in mid-Cretaceous amber. Photograph: Taiping Gao/Nature Communications

(https://isabellevea.files.wordpress.com/2021/03/screenshot-2021-03-04-at-08.55.21.png)

From The Guardian : <u>https://www.theguardian.com/science/2019/dec/10/dinosaurs-had-feathers-ruffled-by-</u> parasites-study-finds (https://www.theguardian.com/science/2019/dec/10/dinosaurs-had-feathers-ruffled-byparasites-study-finds)

A day later, I received an email from my former Ph.D advisor, David Grimaldi. He forwarded to me the original article and asked my opinion on the study because the specimens strangely looked a lot like scale insects to him as well.

Gao et al. (2019) described new insects in amber covering fragments of dinosaur feathers and through their interpretation of the morphology, concluded that they were parasites feeding on dinosaur feathers, related to lice. The interpretative drawing shows an insect with chewing mandibles holding onto feathers.

**There were important problems about this discovery to me.** The identification of the insect was not correct and most importantly, it garnered mainstream media attention. We thought that a quick

response was necessary to point out the mistake. A couple of days later, I was back at the American Museum of Natural History after a few years, and was going through Burmese amber pieces for additional scale insect nymphs. In the meantime, we received additional high resolution photos of the described "lice" from the authors, which allowed to confidently confirm that they were scale insects.

**Scale insects nymphs are commonly found in amber deposits, and this is not surprising.** This developmental stage is the dispersing one, and scale insect nymphs do it so well that they are also coined "crawlers". Scale insects crawlers can be found everywhere on a plant and the soil as soon as there is an infestation. So given the fact that amber inclusions are made of tree resin falling on the ground and catching insects on its way, crawlers are very likely to end up inside amber.

**But why didn't Gao et al. identify them as such?** The main reason is that fossil scale insect nymphs are overlooked and not often described. And this is because the way scale insect systematics was built up. Coccidologists identify and describe extant scale insects using in majority adult female morphology. Also, staining of the insect skin and slide preparation are necessary to provide a description of the setae and secretion pores. By contrast, fossil scale insects are very often found as adult males (which are also mobile and completely different from the females). Although more challenging because slide preparations are not possible, they can still be described because of the exceptional preservation amber allows. Then finally, scale insect nymphs are minute and even though we have descriptions for some of them, it is not easy to examine and describe them at the species level, unless we have also collected adult females. The same situation goes for amber pieces, isolated scale insect juveniles, without an adult stage in the same piece, are usually not described.

After discussing how we would present evidence that the insects in Gao et al. 2019 are indeed scale insects, we drafted a response and submitted it to Nature Communications, 9 days after the original articles was published. It would take over a year of reviewing and communicating with the journal's editor to finally have it accepted. **In the meantime, over the span of one year, Gao et al. 2019 was cited 14 times in other studies published in scientific journals.** 

#### Why are they not dinosaur feather parasites?

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(https://isabellevea.files.wordpress.com/2021/03/nat-comms-reply-fig01.png)

Figure from Grimaldi and Vea 2021. A-B: previously published photo and drawing of scale insect nymphs. C. Photo of a specimen from the AMNH collection taken by David Grimaldi. D-G: Photos and drawings from Gao et al., 2019

#### Mouthparts

The major misinterpretation from Gao et al, 2019 is the mouthpart description. The interpretative drawing (Figure below) is not realistic in terms of mouth position. Lice (the group they assigned the fossil to) mouthparts are not positioned ventrally, but are normally extending forward of the head. But on the fossil specimens, the mouthparts position is indeed ventrally, which is characteristic to the mouth location of Sternorrhynchan insects, a subgroup of the Hemiptera order that have sucking mouthparts located very ventrally, usually between the first pair of legs. Sternorrhynchans are strictly feeding on plant sap and include scale insects.



(https://isabellevea.files.wordpress.com

/2021/03/screenshot-2021-03-04-at-10.28.16.png)

Figure 2 in Gao et al., 2019

The authors also interpreted the mouthparts as chewing type, but none of the specimens examined show clear chewing mandibles. A photo on their publication actually shows external mouthparts that contain a sucking stylet – similar to a straw, as found in scale insects . In one of the specimens, that was drawn by the author, we can see the stylet coiled in the body (See on Figure D for their fossil specimen and A for a comparison with a living scale insect nymph).

#### Legs

To justify the feeding behavior on dinosaur feathers, Gao et al interpreted the fossil single claw on the insect leg as grasping claws on feathers. However, these claws are not specialised in the way we find in lice species. Scale insects are uniquely characterised with legs carrying a fine single claw (as opposed to two claws in other insects). This claw also bears two long small extensions that are called digitules and they are visible on the photos of one of the specimens they published (on the Figure, see G "setae" label on the leg, and B and C for comparison).

#### Head thorax abdomen separation

A characteristic of scale insect nymphs is the absence of head-thorax-abdomen separation, a feature that is retained in adult females. The fossils clearly do not show constrictions although the interpretative

drawing exaggerated the separation between head thorax and abdomen. Lice do have well separated body parts.

#### Why were scale insect nymphs on dinosaur feathers?

As mentioned previously, scale insect nymph are common in amber because they were easily captured by resin leaking out of tree trunks. Feathers can also be captured by resin falling on the ground. So it is not surprising to see a co-occurrence of these two in the same amber pieces that is coincidental.

#### I know, not as sensational as dinosaur parasites.

#### References

Gao, T., Yin, X., Shih, C. *et al.* New insects feeding on dinosaur feathers in mid-Cretaceous amber. *Nat Commun* **10**, 5424 (2019). https://doi.org/10.1038/s41467-019-13516-4 (https://www.nature.com/articles /s41467-019-13516-4.pdf)

Grimaldi, D.A., Vea, I.M. Insects with 100 million-year-old dinosaur feathers are not ectoparasites. *Nat* <u>Commun 12, 1469 (2021). https://doi.org/10.1038/s41467-021-21751-x (https://www.nature.com/articles</u> /s41467-021-21751-x.pdf)

▲ imv ► publication, scale insects ♥ Leave a comment ③ March 5, 2021March 5, 2021 4 Minutes

## Natural History of scale insects in 1734





*First chapter of Memoire pour servir a l'histoire des insectes. Description of scale insects and cochineal. You can see on the drawing cacti, host plants of the cochineal Dactylopius. Source gallica.bnf.fr / BnF* 

One of my favorite times when I was studying in Natural History Museums in Paris then New York, was to roam in their libraries. They house some of the oldest books on natural history. Despite today's easier and instant access to electronic version of publications, pre-internet publications were often only available in library aisles, waiting sometimes decades for a curious of naturalist to find them. This was my favorite time: looking for a publication on scale insects, going to the library, retrieve the precious printed copy and turn the pages slowly. I love old libraries, the smell, the almost romantic feeling that radiates from them.

I only recently came across an old French book from 1734 that describes the natural history of insects. Two sections of this multi-tome memoire is dedicated to scale insects . At the time, the name that we use in French now for scale insects: cochenille, was only used for the Mexican cochineal, making red dye. All other scale insects were called "gallinsectes" as they resemble parts of plants for the most derived groups.

Here is the pdf of the book, scanned from the French National Library: <u>http://gallica.bnf.fr/ark:/12148</u>/<u>bpt6k65246505\_(http://gallica.bnf.fr/ark:/12148/bpt6k65246505)</u>

This book describes the biology and what we knew about scale insects back in 1734. For me, reading old books like these gives me some appreciation about how much was known before, how scientists/naturalists perceived nature and diversity, and gives a sense of the pace in research, how meticulously observant naturalists were and that they took time to look around the world even though the tools were limited.

Unfortunately, today, we cannot work at this slower pace anymore, and this makes me think that we don't have time anymore to slow down and think more deeply about phenomema and processes that we are trying to understand in a deeper level. Or simply observe the world with more appreciation.

**i**mv **b** Uncategorized

# Old collection scale insect slides and the power of confocal microscopy

Following a tweet I saw today on Plecoptera slide collection, I became nostalgic of scale insect collections.

Last week <u>@bugsymac1 (https://twitter.com/bugsymac1?ref\_src=twsrc%5Etfw)</u> spent some time working on the <u>@NatSciNMS (https://twitter.com/NatSciNMS?ref\_src=twsrc%5Etfw)</u> Plecoptera collection, including hunting for missing type specimens! <u>#Stoneflies (https://twitter.com/hashtag/Stoneflies?src=hash&</u> <u>ref\_src=twsrc%5Etfw)</u> <u>#Entomology (https://twitter.com/hashtag/Entomology?src=hash&</u> <u>ref\_src=twsrc%5Etfw)</u> <u>pic.twitter.com/ynzlBaKQCR (https://t.co/ynzlBaKQCR)</u>

— Ashleigh Whiffin (@AshWhiffin) <u>April 16, 2018 (https://twitter.com/AshWhiffin/status /985878199623143425?ref\_src=twsrc%5Etfw)</u>

Scale insect descriptions and identification are done using mounted material of stained insect cuticle. Because both males and females don't have very sclerotized cuticle, they become transparent and outer structures are very easy to observe with a light compound microscope.

An example here, we have wax scale insects on a leaf (top left). After removing the inside to keep only cuticle for staining and mounting, we can have a better view of micro structures (top right). This helps making descriptions illustrated by diagrams (bottom).



(https://isabellevea.wordpress.com/2018/04/16/old-

collection-scale-insect-slides-and-the-power-of-confocal-microscopy/screen-shot-2018-04-16-at-19-38-14/)



(https://isabellevea.wordpress.com/2018/04/16/old-collection-scale-insect-

slides-and-the-power-of-confocal-microscopy/screen-shot-2018-04-16-at-19-38-24/)



(https://isabellevea.wordpress.com/2018/04/16/old-collection-scale-

insect-slides-and-the-power-of-confocal-microscopy/screen-shot-2018-04-16-at-19-38-33/)

In the past, male samples were not mounted or if they were, the quality of mounts was not as good as female samples. This is mostly because males being ephemeral, they were not used for identification or description. There are however quite a few old slide mounted males in collections and especially for species that are difficult to find, they are in my opinion, quite valuable to study for phylogenetic purposes.

When I was working on a phylogenetic study of scale insects, I spent a lot of time examining male samples in old collections. Knowing that males are so rare compared to females, finding an old slide mounted male of a rare species was like finding a treasure.

For a couple of years, I focused on fossils and male morphology of the ensign scale insects (Ortheziidae). So I found slide mounts for Ortheziidae males but some of them couldn't be observed clearly because the cuticle was not cleared properly. Using a traditional light compound microscope, it is just impossible to look at details of setae and secretion pores.

During my master's degree, I worked in the Natural History Museum in Paris, and my supervisor showed how to unmount samples, and reprocess them for clearing and staining. But these Ortheziidae males were rare so I tried another alternative: confocal scanning laser microscopy.

So here it is:



(https://isabellevea.wordpress.com/2018/04

FIGURE 1. Dorsal surface of *Graminorthezia graminis* (Tinsley). **A.** Compound light microscope. **B.** Confocal microscope. Scale bar: 500 µm.

/16/old-collection-scale-insect-slides-and-the-power-of-confocal-microscopy/screen-shot-2018-04-16-at-19-56-34/)



(https://isabellevea.wordpress.com/2018/04/16/old-

FIGURE 6. Ventral surface of *Orthezia annae* Cockerell. **A.** Light compound microscope. **B.** Confocal microscope.

collection-scale-insect-slides-and-the-power-of-confocal-microscopy/screen-shot-2018-04-16-at-19-57-03/)

A lot of surface structure were easily revealed at 633 nm. This is not surprising as normally, staining is made with fuchsin acid, its emission wavelength is 630 nm.

So how much detail can we actually see?

These are abdomens of mounted samples. Setae and secretion pores are easy to see.



When you look more closely, it is actually quite incredible how much detail can be captured!



I think confocal imaging is a great non-invasive option to work on old collection samples, especially ones that are slide mounted, obscured and rare. Although not every place has a confocal microscope (especially museums), the American Museum of Natural History is equipped with a Zeiss LSM710 which was really handy!

Do you know any confocal images of old collection slides? I would love to see them!

#### References

*Ceroplastes* photo source: <u>IDtools.org (http://www.idtools.org/id/scales</u>/gallery\_index.php#prettyPhoto)

Confocal images from <u>Vea (2015) (http://digitallibrary.amnh.org/bitstream/handle/2246/6551/N3812-highres.pdf?sequence=2&isAllowed=y)</u>.

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Leave a comment

## News about my research: Evolution2018conference and E93 manuscript available on BiorXiv

Hello everyone!

Spring is (almost) in Edinburgh, and here are some news on my research activities!

This is an update that I will be presenting a talk at <u>Evolution 2018 (http://evolutionmontpellier2018.org/)</u> in Montpellier this summer in the symposium *S-35 Combining fossils and phylogenies in studies of diversification* organised by Fabien Condamine and Daniele Silvestro.

Combining fossils and living species to better understanding scale insect evolution was the main work of my Ph.D but even if I have since switched my research activities, I intend to continue on working on this project as there is still more data to acquire! Getting a more stable dated tree of scale insects will help to answer questions on their evolution and adaptation.

Additionally, I just submitted a manuscript on mealybug female neoteny and E93 transcription factor with links to the juvenile hormone. This work was conducted at Nagoya University where I am still collaborating on examining mealybug metamorphosis in the context of the juvenile hormone. <u>You can find a preprint on BiorXiv. (https://www.biorxiv.org/content/early/2018/03/16/283556)</u>

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## Insect-fungus symbiosis: a scale insect feeds her own roof!

Scale insects are often associated to fungus. Females secreting honeydew trigger sooty mold growth and harm the host-plant. Induced undesirable sooty mold on plants makes scale insects some of the most damaging phytophagous insects. For instance, *Cryptococcus fagisuga*, a felt scale insect living on beech trees is associated to a fungus called *Neonectria coccinea* that will grow from the holes created by the insect's puncture hole from which it feeds. Ultimately, the mold growth becomes more lethal to the tree than the insect. This is so true that I remember my Master mentor telling me that beech trees have almost disappeared from Paris because of this species!

Sooty mold are also harmful to scale insects, as the female secreting honeydew are sessile and they

would literally die in their own moldy poop. This is when ants comes into play as essential mutualistic animals: by profiting from scale insect honeydew droplets as a food source, ants naturally clean their secretive waste, preventing sooty mold growth.



*O. machinator* made of fungus



Fungus hyphae



The fungus and mealybug have almost fused! arrows show fungus hyphae inserted in the mealybug cuticle

Images showing the fungus structure in which the mealybug lives in (left) and details of fungus mealybug interaction (right) . Source: modified from Gavrilov (2017)

In an extraordinary situation, a species of scale insects from the mealybug family (Pseudococcidae) has taken advantage of mold and developed a unique symbiosis with a fungus species. *Orbuspedum machinator*, a newly described species from bamboo twigs of Thailand, has legless females and uses hyphae of a fungus *Capnodium* species to make herself a roof. In exchange, the mealybug secretes honeydew and feed the fungus.

A win win situation.



*Hypothetical scheme of the formation of a fungal domicile by Orbuspedum machinator gen. et sp. nov.* (*Gavrilov* 2017)

#### Reference

Open access: Gavrilov (2017) <u>An amazing symbiosis between an animal and a fungus in a new species of legless mealybug (Insecta: Pseudococcidae) (http://www.tandfonline.com/doi/full/10.1080 /00222933.2017.1365180)</u>

## Manuscript self-archiving #1: many questions!

Hello all!

If you have read the <u>Preprint (https://isabellevea.wordpress.com/preprints/)</u>, you know that I am trying to act and become 100% open access with my manuscripts. I made a list of the publications with links to the PDFs available online without a paywall (if the paper is open access or available in other websites) but there are still a few publications that require a subscription to the journal.

I thought it would be interesting to write about my "becoming open access" process as it seems that it will be tricky (but I am sure feasible) for a few reasons:

1- Which version of the manuscript is acceptable to make a preprint of when the manuscript has already been published?

- Should I use the first submission version (but sometimes major changes have been done before the second submission)?
- Can I make a preprint of the resubmission (without the publisher's proofreading)?
- Or can I make a preprint of the last version of the manuscript before they prepared the layout?

2- Are there any publishers that do not allow at all to have a preprint version of my work after I published it?

For instance, what does that mean??

#### Assignments of copyright

1) The Author(s) assigns to *Magnolia Press* exclusive copyright and related rights in the Article, including the right to publish the Work in all forms and media including print and all other forms of electronic publication or any other types of publication including subsidiary rights in all languages.

Please don't judge me for signing things I don't understand, I was a naive Ph.D. student that wanted to have a publication (Okay you can judge me....)

HELP!



## New research life in Edinburgh: fieldwork in California, blogging about nature crazy sex life

### and more



Vine mealybugs in North California vineyard

#### Hello everyone,

A lot has happened since I moved to Edinburgh. Here are the main updates:

- I am currently carrying out fieldwork in California until May, and looking for obscure mealybugs with B chromosomes. Follow me on Twitter!
- You can find now <u>a new blog on crazy sex determination systems (https://medium.com/how-we-do-sex)</u>, with monthly contents. And there is a <u>French version (https://medium.com/cest-une-fille-ou-un-gar%C3%A7on)</u>!

- In June and July, I am very excited and honoured to be attending <u>the outstanding 100-year-old</u> <u>embryology course (http://www.mbl.edu/education/courses/embryology/)</u> at Woods Hole. You will likely hear more about it either on Twitter or here.
- In August, you will see me at <u>ESEB2017 (http://www.eseb2017.nl/)</u> in Groningen, where I will present my work on scale insect adult metamorphosis.
- Leave a comment ③ April 14, 2017April 20, 2017 ≡ 1 Minute

## Move to Edinburgh

Hello everyone!

This is an update that I just moved to Edinburgh in January 2017 to start a Marie Sklodowska Curie Fellowship at the University of Edinburgh. I will be working with Laura Ross on paternal genome elimination in scale insects and B chromosomes!

For more information on this project: <u>http://cordis.europa.eu/project/rcn/202882\_en.html</u> (<u>http://cordis.europa.eu/project/rcn/202882\_en.html</u>)

Leave a comment ③ January 24, 2017 January 24, 2017 ≡ 1 Minute

### Why should biologists use GitHub?



from GitHub repository <u>here (https://github.com/zourloubidou/Coccomorpha-divergence-time/blob/master</u> /<u>Plots.md</u>)

Today, all biologists use computers on a daily basis, produce and analyse data. A lot of us now have to learn programming (even just some bits of it).

I am not a computer scientist/engineer, and I am far from a bioinformatics person (yet), but I have started to discipline myself to make my published research as reproducible as possible and this is not only by depositing DNA sequence data to NCBI, but also analysis pipelines and command lines made available to the public on GitHub.

GitHub is a great public repository hosting service for publishing programming source code, but it can also be used to detail your analysis pipeline and code, and even create tutorials on softwares or pipelines for others.

For example, the <u>Trinity tutorial from Brian Haas (https://github.com/trinityrnaseq</u>/<u>/KrumlovTrinityWorkshopJan2016/wiki/Home/e67c7a4ae4fe005866a56371ea29f15c79e8ccfb)</u> was a life saver for a biologist like me that had never touched any next-generation sequencing data.

As a biologist, to support my recent publication on scale insect phylogenetics, I created a GitHub

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<u>repository (https://github.com/zourloubidou/Coccomorpha-divergence-time/blob/master/divergence-time.md)</u>that details all the steps and command lines in MrBayes and R analyses. This provides transparency to the reader and more rapid reproducibility.

Tip: If you are worried about your analysis pipeline or data being online during the manuscript review process, academic researchers can apply for free space for 5 private repositories. For more information, <u>check here (https://github.com/blog/1840-improving-github-for-science)</u>.

Nowadays, a lot of biologists will come to work in a multidisciplinary environment, and it implies learning new skills. In bioinformatics in particular, workshops are available but the internet is a great resource to learn skills by ourselves and GitHub can help both learning how a software works, but also making the details of informatics methods available to other biologists that are also learning how to use these softwares (from command lines for *de novo* assembly using Trinity or making a simple plot with R).

Leave a comment ③ May 8, 2016 ≡ 1 Minute

## SICB2016: Society for Integrative and Comparative Biology meeting

Kicking off 2016 with the SICB annual meeting which is taking place in Portland and starting on January 3rd.

I will be presenting poster and oral presentations related to my postdoctoral work on the hormonal regulation of extreme sexual dimorphism in scale insects.

This is my first SICB meeting and I am excited to meet people working on other taxonomic groups than insects!

Follow #SICB2016 on Twitter for live updated of the meeting.

#SICB2016 Tweets (https://twitter.com/hashtag/SICB2016)

Leave a comment ③ January 2, 2016May 8, 2016 ≡ 1 Minute Isabelle M. Vea

Blog at WordPress.com.