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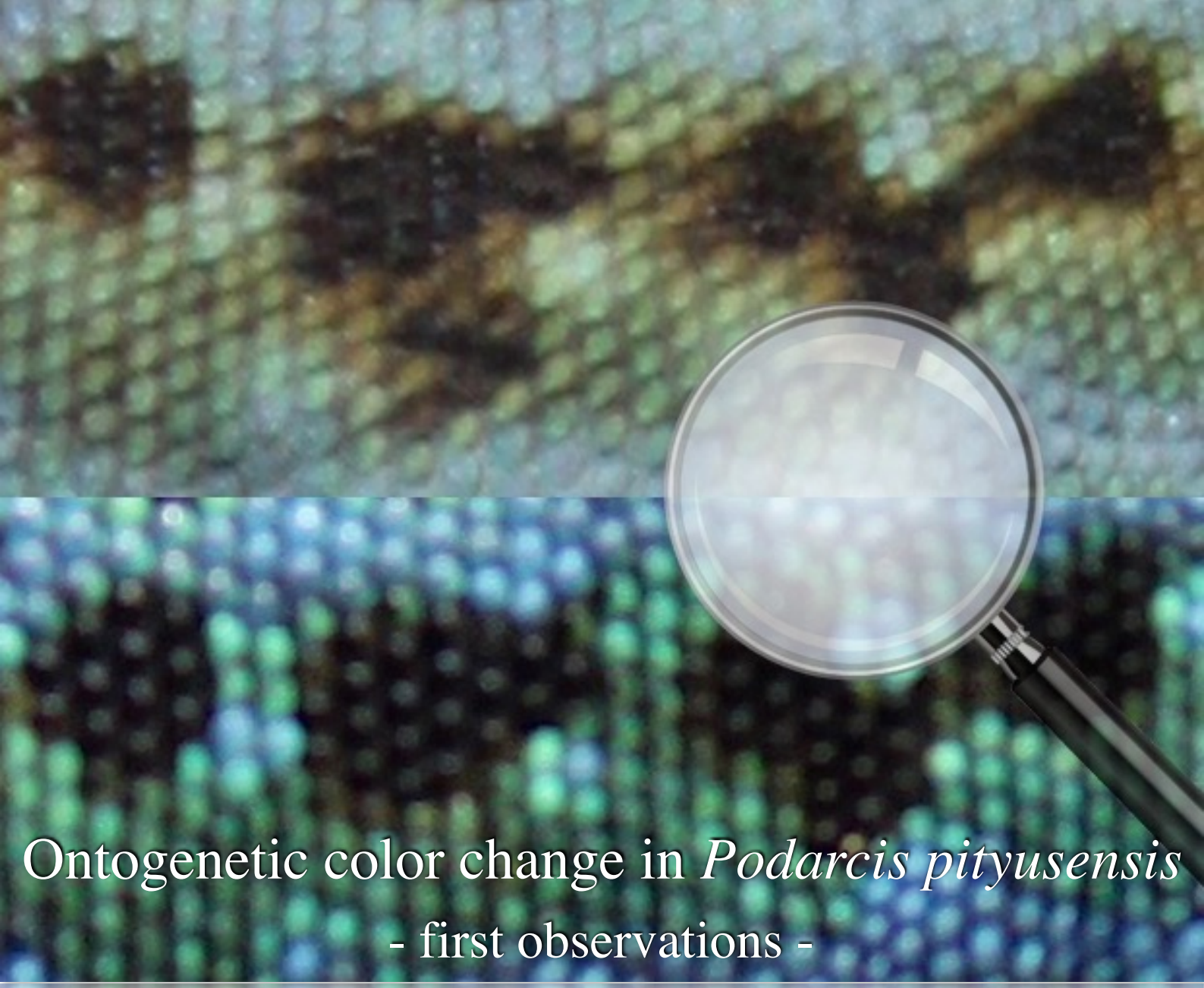


Ontogenetic color change in *Podarcis pityusensis*
- first observations -

MARTEN VAN DEN BERG, MIKE ZAWADZKI and MICHAEL KRONIGER, March 2016

Abstract

A short introduction and a hypothetical model concerning color formation in lacertid lizards is presented. Thereafter we observe and discus the ontogenetic changes in a single female captive bred *Podarcis pityusensis* lizard from Formentera (Balearic islands/Spain). Striking transformations concerning coloration are observed.

Keywords: Ontogenetic color change, *Podarcis pityusensis*, Formentera.

Introduction

Being in the possession of a small group of legal captive bred lizards of a few *Podarcis pityusensis* subspecies, offers the possibility to study topics, which are almost impossible to study under "in the field" conditions. Amongst them are the individual changes of a single lizard, during the development from hatching until adulthood. To study this topic on site, would require multiple recaptures within a small period of time. It is conceivable that this is possible, but not very practical.

In this case, we will follow a female lizard, with ancestors originating from Formentera (Balearic islands/Spain), in her development of coloration during the first year of her life. But before we can interpret what we are observing, we first need some understanding on how coloration is accomplished in such lizard. This might constitute a small problem, because almost no research regarding color formation has been undertaken in lacertid lizards.

Therefor we first have to establish a hypothetical model, based on what has become known in some other lizard families (TAYLOR & HADLEY 1970 ; KURIYAMA et al. 2006 ; SAENKO et al. 2013 ; TEYSSIER et al. 2015). In addition, we have to remember that the process of vision is nothing more then building a representation of the surrounding environment in the optical parts of the brain, fed by information gathered in the eyes by a large number of photoreceptor cells, which will translate the information of the absorbed wavelengths into an action potential send to the optical parts of the brain.

When we, as human observers, are looking at a lizard, we perceive a different image of that lizard compared to what a congener lizard would perceive. Also the perception by other animals, such as terrestrial or avian predators, differs from what we, or an other lizard would see. This is all due to the differences in photoreceptor cells and their visual pigments. We recommend BOWMAKER (1998) for an introduction and overview of the evolution of color vision in vertebrates. One of the most appealing differences between our color vision, and the color vision in lizards, is their ability to perceive wavelengths in the ultraviolet range of the spectrum (PÉREZ I DE LANUZA & FONT 2014).

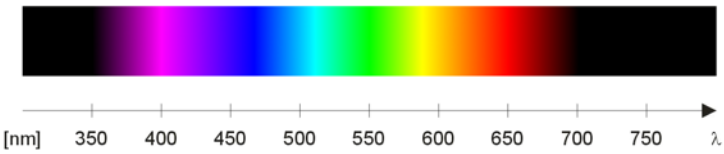


Fig 1. The human visible spectrum (wavelengths in nm).

So, when we are looking at a lizard, we are processing an image of that lizard in our brain, by the received wavelengths of light, originating from sunlight, selectively "reflected" by the skin of the lizard. This "reflection" of light occurs in the outermost cells of the skin, collectively called chromatophores. In lizards, like other reptiles and amphibians, two different mechanisms of light "reflection" or color formation are present: Pigment color and structural color.

Pigment color is achieved in chromatophores with organelles containing pigments. These pigments are substances that have a color resulting from selective color absorption, which is the same for all viewing angles.

In reptiles two classes of pigments are present. First the yellow, orange and red pigments, found in the so called xanthophores and erythrophores. These pigment cells are usually present in the outer layer of the dermis, just underneath the epidermis. The pigments found in reptile xanthophores and erythrophores are belonging to either the carotenoids or pteridines or both (TAYLOR & HADLEY 1970 ; SAENKO et al. 2013 ; TEYSSIER et al. 2015). In lacertid lizards these pigments are belonging to the carotenoids (FITZE et al. 2009 ; PEEK 2011), and consist of lutein, zeaxanthin, astaxanthin, and canthaxanthin (FITZE et al. 2009).

The second class of pigment in reptiles is the dark-brown to black eumelanin pigment, found in the so called melanophores. These pigment cells are usually arranged in close relation to iridophores (structural color creating cells), underneath the xanthophores and erythrophores, where they contribute in color formation. In the deeper parts of the skin, melanophores form a protective basal layer, protecting from harmful UV radiation. Sometimes smaller melanophores are also present in the epidermis, where they play their role in the formation of dark patterns.

Blue pigment (in pigment cells called cyanophores) is absent in reptiles, however TEYSSIER et al. (2015) did observe such blue pigment of unknown origine in chameleons.

Structural color is created in chromatophores called iridophores. Inside these iridophores in reptiles, platelets of guanine crystals interfere with the passing light, resulting in a selective "reflection" of some part of the spectrum. Actually this is a much more complex process than reflection, and is called light interference. The orientation and thickness of the guanine platelets and distance between platelets is determinative for the "reflected" wavelengths (KURIYAMA et al. 2006 ; SAENKO et al. 2013 ; TEYSSIER et al. 2015).

In contrast to pigment based chromatophores, the viewing angles in iridophores is causing differences in the color perception. This phenomenon is often observable while watching a moving lizard under bright sunlight.

Because in lacertid lizards little is known on the arrangement of the chromatophores, besides an early and inconclusive study on *Podarcis bocagei* (BOWKER et al. 1987), we have to hypothesize how this in lacertid lizards is organized. Deducing from the results in other lizards (KURIYAMA et al. 2006 ; SAENKO et al. 2013 ; TEYSSIER et al. 2015), we were able to construct the following model:

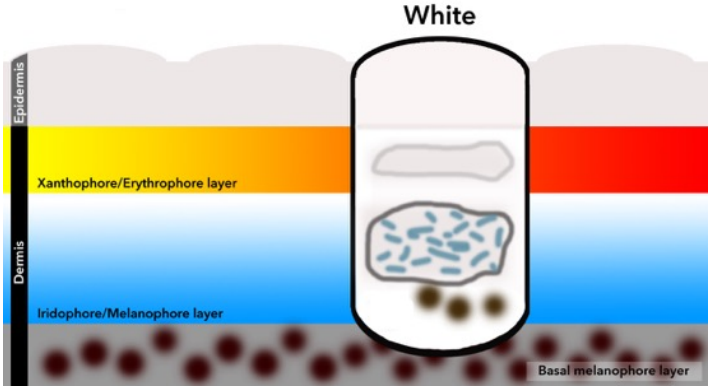


Fig 2. The white chromatophores arrangement.

White could be created by iridophores with unorganized guanine crystals, which are "reflecting" multiple wavelengths in various directions, what we perceive as white light. Pigment cells in the xanthophore and erythrophore layer are absent, or present, but then without containing much pigment.

Melanophores should be situated underneath the iridophores, without having much influence. When the eumelanin pigment containing organelles are stretching out beside or above the iridophore, a darkening effect should be the result.

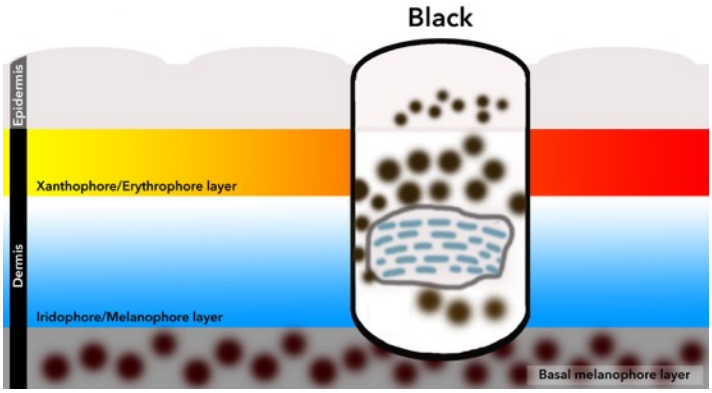


Fig 3. The black chromatophores arrangement.

Black is caused by absorption of most parts of the incident light, caused by the eumelanin pigment inside the melanophores. From the presence of dark spots in the shedded skin, we can conclude that small epidermal melanophores might be present in lacertid lizards, like they are in *Plestiodon* (KURIYAMA et al. 2006). The iridophores are containing blue "reflecting" photonic crystals, because we still can observe this blue in most black parts, when we exaggerate the saturation of the blue channel in our photo editing software.

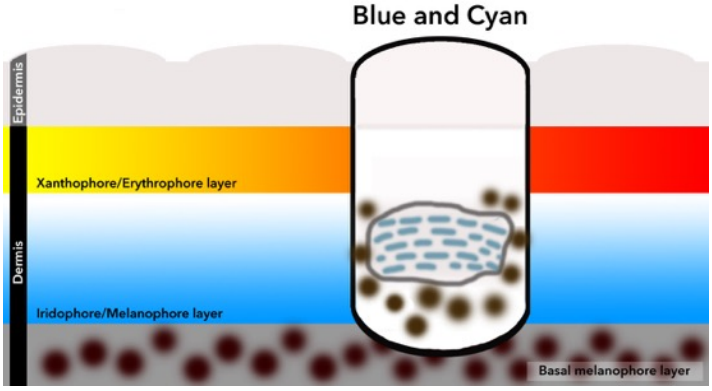


Fig 4. The blue and cyan chromatophores arrangement.

Blue and cyan are both structural colors originating from light interference inside the iridophores, with organized guanine crystals, where in the case of cyan, the photonic crystals are a little more wider apart, compared to the blue situation. Darkening is achieved by the degree of eumelanin pigment in the

melanophore, which might stretch out beside or above the iridophore. More pigment containing melanosomes will result in a darker blue, or a darker cyan.

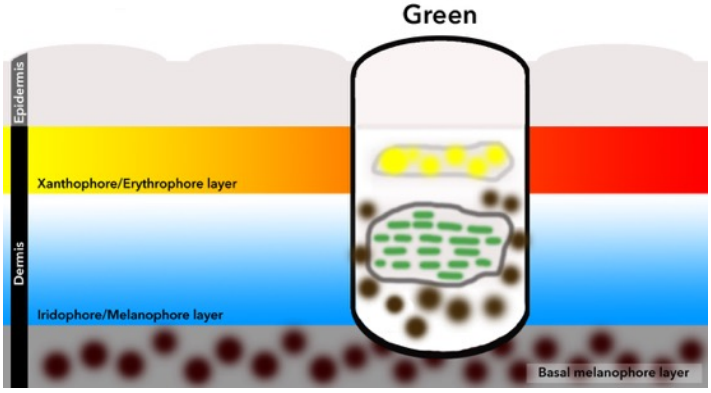


Fig 5. The green chromatophores arrangement.

Green is also a structural color, originating from light interference inside the iridophores, with organized guanine crystals, where the photonic crystals are a little more wider apart, compared to the cyan situation. Probably are these "green reflecting" platelets in close relation to the presence of "cyan reflecting" platelets. Yellow pigment in the above xanthophore layer is therefor necessary, to absorb the bluish wavelengths, consequently showing only the green coloration (MILINKOVITCH 2015). This yellow pigment could additional enhance the brightness of the green coloration.

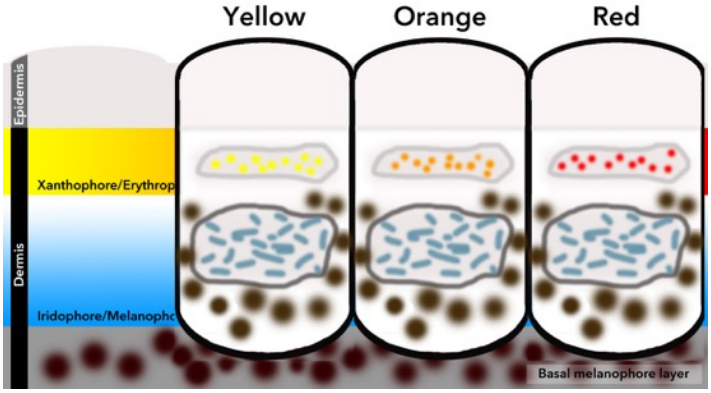


Fig 6. The yellow, orange and red chromatophores arrangement.

Yellow, orange and red coloration is created by the pigment in the xanthophores and erythrophores. The degree of concentration of the pigment seems to be responsible for the hue of the reflected color. Low concentration of pigment leading to yellow, and high concentration of pigment resulting in red. The underlying iridophores contain unorganized guanine crystals, acting as structural white. This white background enhances the brightness and visibility of the yellow, orange and red coloration of the pigment cells.

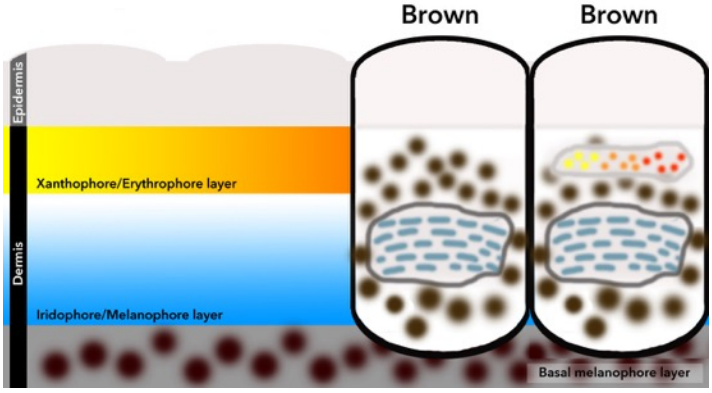


Fig 7. The brown chromatophores arrangement.

Like in *Plestiodon* (KURIYAMA et al. 2006) and *Phelsuma* (SAENKO et al. 2013), brown coloration is caused by melanophores arranged above the iridophores with organized guanine crystals. However, we think that also unorganized guanine crystals could be associated with brown coloration, because in some cases in *Podarcis pityusensis* brown coloration is just resembling a range of gray shades. In other cases we observe in brown parts yellow and red colors too, suggesting that pigment containing xanthophores and erythrophores are present.

This color formation model for lacertid lizards was presented by us at the annual meeting of DGHT-AG Lacertiden in Gersfeld/Rhön (Germany) on saturday the 12th of March, 2016. During the presentation this model has been explained in depth, with additional examples. A video of the presentation is available at www.lacerta.de.

Fig 8. Presentation video available from 13.03.2016.

Fig 9. *Podarcis pityusensis formenterae* hatchlings, a few days old (24.08.2012), belonging to the same clutch as our study specimen.

Material and methods

Our study specimen is a captive bred, female *Podarcis pityusensis*, with ancestors originating from Formentera (Balearic islands/Spain), which hatched on 22.08.2012. In the first stage, she will be compared to an unidentified female from the same clutch. We compared lateral and ventral images, taken with a Canon EOS 500D camera, equipped with a Canon EF-S 18-55 mm f 3.5-5.6 IS lens, under natural sunlight conditions.

Three stages were recorded, respectively at the age of approximately 2 months (13.10.2012), at the age of approximately 7 months (28.03.2013), and at the age of approximately 1 year (31.08.2013). In the case of color comparison by removing or enhancing color channels, we used the standard saturation settings in Adobe Photoshop Elements 10.

In order to achieve a better understanding of how the colors are arranged in the study specimen, we

Fig 10. *Podarcis pityusensis formenterae* hatchling, born on 22.08.2012, from the same clutch as our study specimen.

Fig 11. Original image of *Podarcis pityusensis formenterae* hatchling.

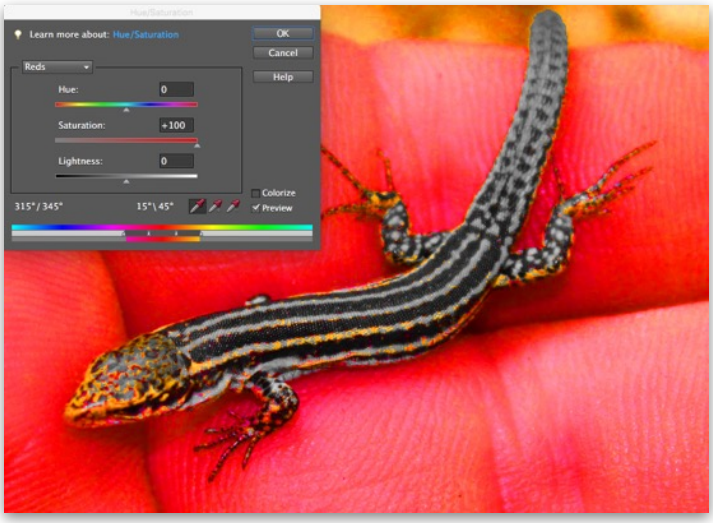


Fig 12. Image of *Podarcis pityusensis formenterae* hatchling, with an exaggeration of the **red** channel.

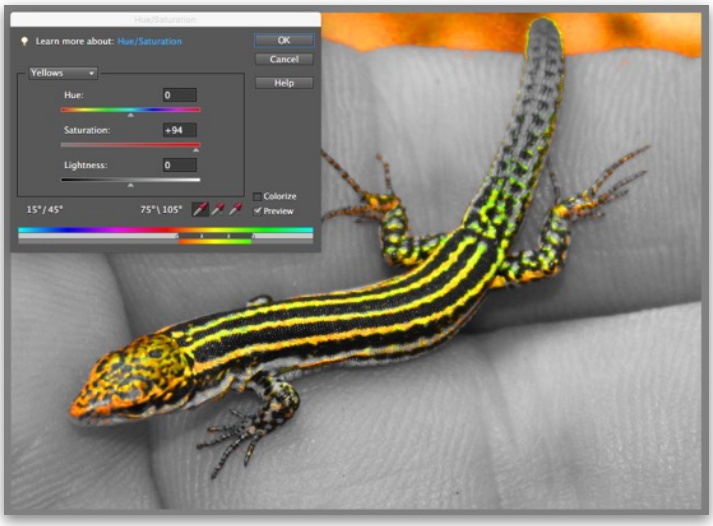


Fig 13. Image of *Podarcis pityusensis formenterae* hatchling, with an exaggeration of the **yellow** channel.

adjusted the saturation levels of the color channels, using the following method: First the saturation level of all six (red, yellow, green, cyan, blue and magenta) were set to -100, resulting in a gray scale image. Then for each color channel, except the magenta channel, which color is not present, the saturation level was set to +100, resulting in an exaggerated image, just for that color channel (see figures 11 - 15). Note that there will be some overlap in adjacent colors.

Initial coloration of a hatchling

When we observe this hatchling in vivo, we see a little colored lizard, with some faint yellowish dorso-lateral lines, and a faint greenish tail, on a dark background. In the original image of the hatchling (Fig 11), this coloration is even less conspicuous.

But when we observe the exaggerated images (Fig 12 and 13) for the channels of the pigment colors red and yellow, it becomes evident that yellow and orange pigments are present on the head, legs, and in the dorso-lateral lines, although in the lower lateral lines there seems to be less coloration. This is probably due to the angle of exposure, resulting in less influence from light interference by the structural white iridophores, arranged underneath the pigment cells.

Blue coloration is found in the darker parts, but only when the blue channel is exaggerated (Fig 14). This seems to be in accordance with prior observations on black parts in these lizards. However, note the apparent absence of blue in the main lateral black band.

The other structural colors, cyan and green, are mainly found in the lighter parts of the tail, and in lesser extent on the legs (Fig 15 and 16). Some green is noticeable in the dorso-lateral lines.

Because both pigment coloration and structural coloration seems to be highly discrete, we presume that in this hatchling the amount of pigments in the pigment cells still should increase, but that does not seem to be the case, and either the organization of the guanine crystals in the iridophores, or the amount of guanine crystals in the iridophores, respectively

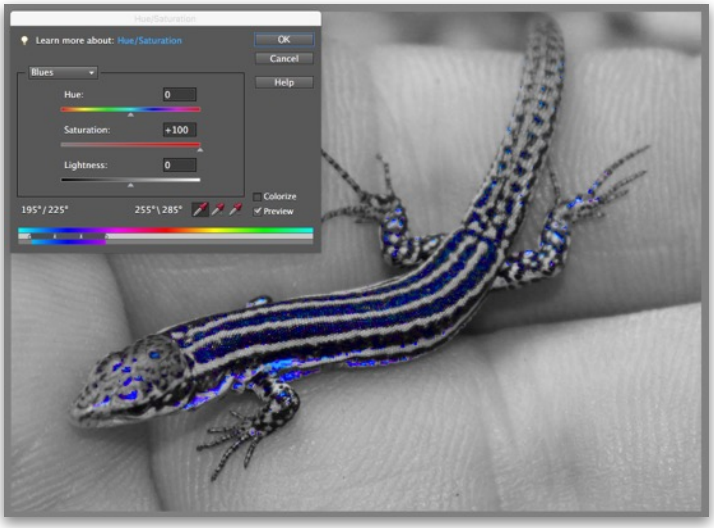


Fig 14. Image of *Podarcis pityusensis formenterae* hatchling, with an exaggeration of the **blue** channel.

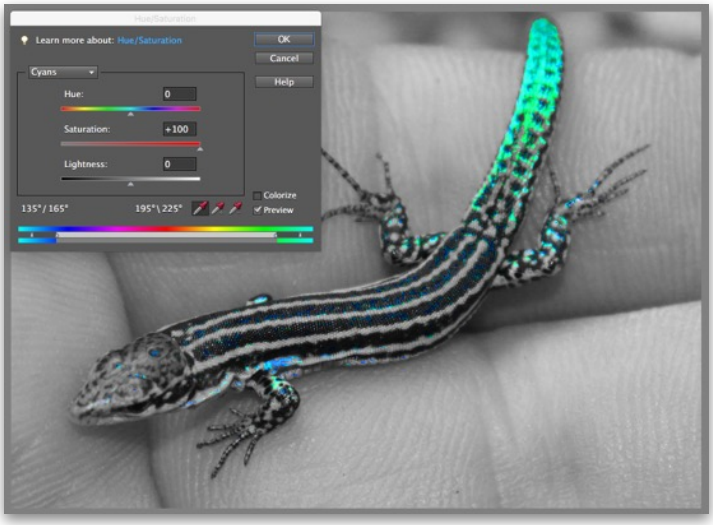


Fig 15. Image of *Podarcis pityusensis formenterae* hatchling, with an exaggeration of the **cyan** channel.

should change, to become more organized, or increase, to become more influential. This might be one of the topics for future research.

Dorsal changes from hatchling until 2 months old

Within the first two months, striking changes can be observed (Fig 17). The central dorsal area (A) is transformed from a regular black line flanked by two faint yellowish lines, to a central green area, with an irregular black pattern. The faint yellowish dorso-lateral line (B) is transformed into a faint cyan color. Both situations require a transformation inside the iridophores, analogous to the above mentioned.

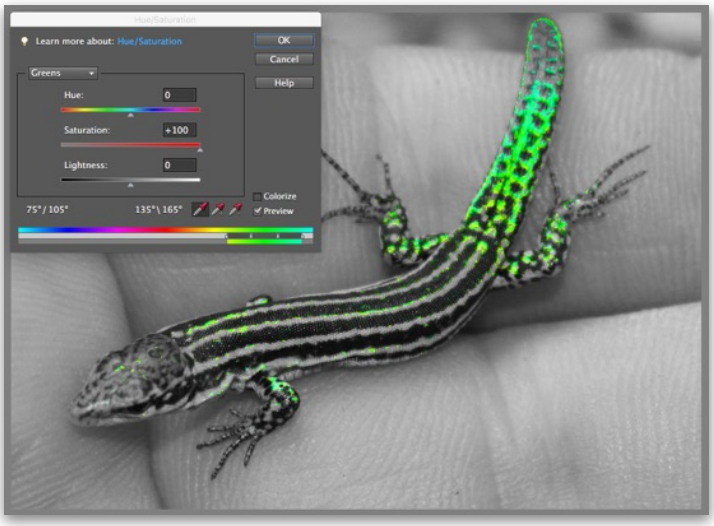
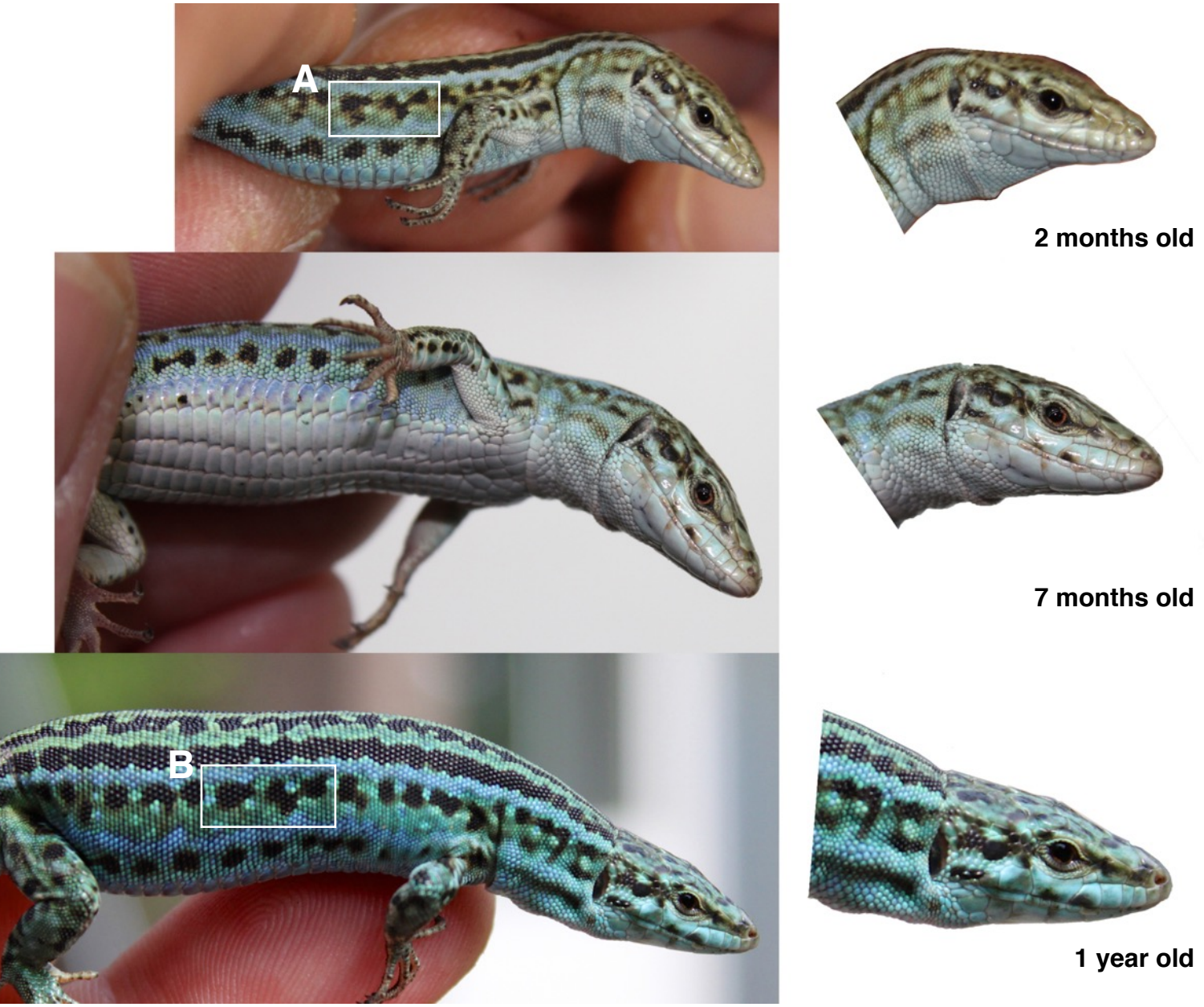


Fig 16. Image of *Podarcis pityusensis formenterae* hatchling, with an exaggeration of the **green** channel.

Fig 17. Comparison of the hatchling and our study specimen, at the age of two months. Striking changes can be observed.



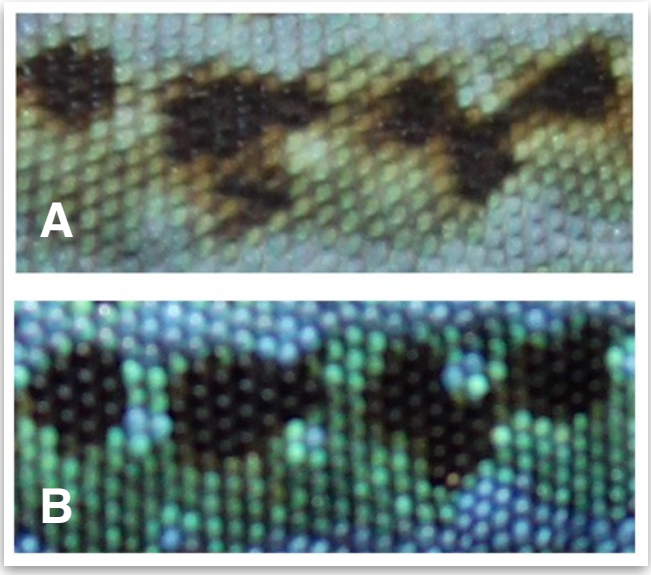


Fig 18. Lateral views from our *Podarcis pityusensis formenterae* study specimen. The outlined areas, A and B, will be compared.

Lateral comparison between 2 months and 1 year

Also the differences in coloration between 2 months and 1 year are quite striking. First the still somewhat faint cyan parts becomes significantly more bluish. Secondly the light green parts are getting darker, and thirdly the dark pattern intensifies, but not on all scales. The lower part of the center black/brown spot (Fig 18 A) transforms into green (Fig 18 B).

When we observe the effects of exaggerating the different color channels, it seems that the pigment colors red and yellow are disappearing (Fig 19 and 20).

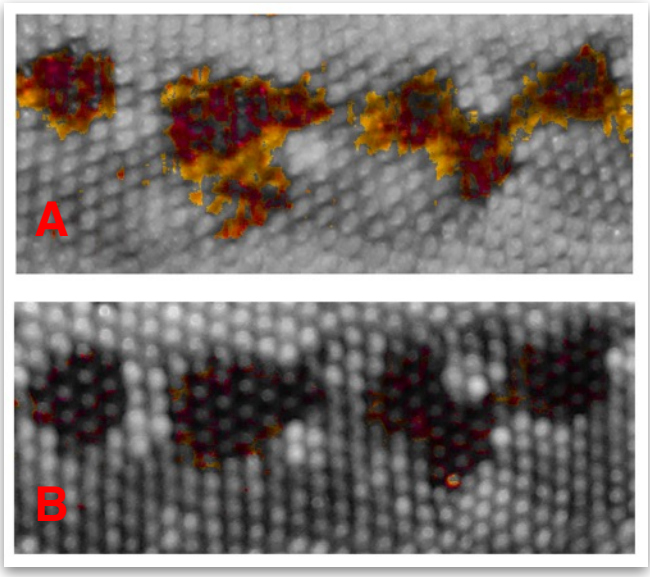


Fig 19. Exaggeration of the red channel.

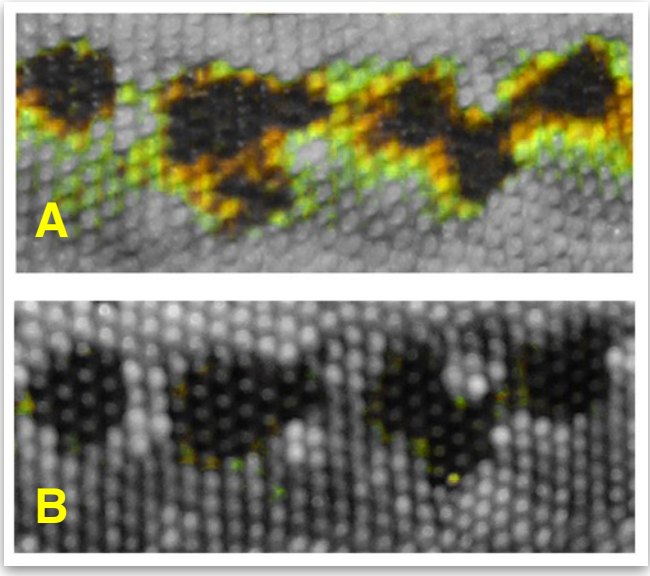


Fig 20. Exaggeration of the yellow channel.

In the blue channel there is actually not much going on, except that in these images (Fig 21), like the hatchling (Fig 14), no blue light interference in the lateral black areas seem to occur. This in contrast to most other black areas.

The cyan and green channels are showing a tendency towards more intense cyan, and a darker green (Fig 22 and 23). Also the transformation of the lower black part of the center dark spot (Fig 23 A) into green (Fig 23 B) is clearly visible.

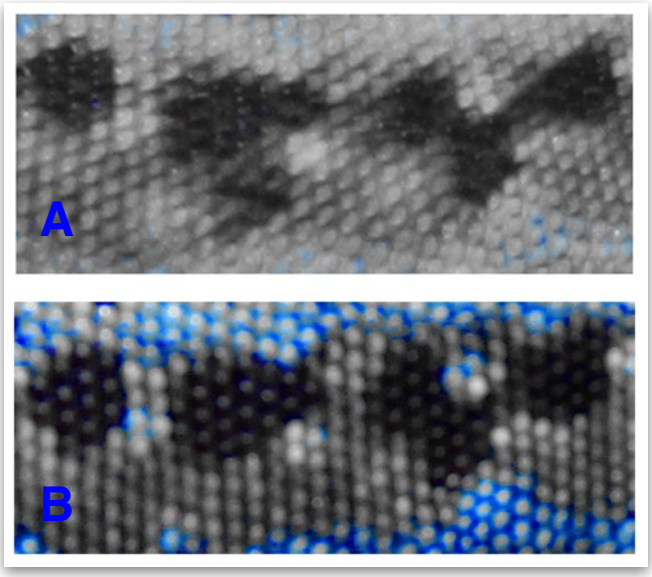


Fig 21. Exaggeration of the blue channel.

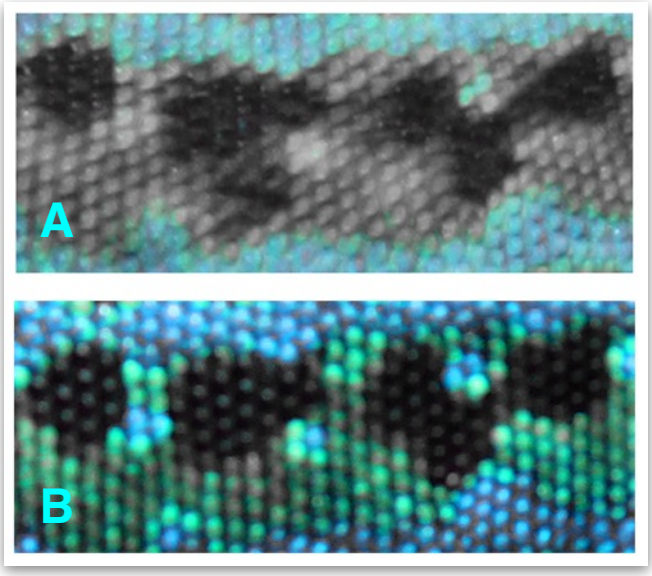


Fig 22. Exaggeration of the cyan channel.

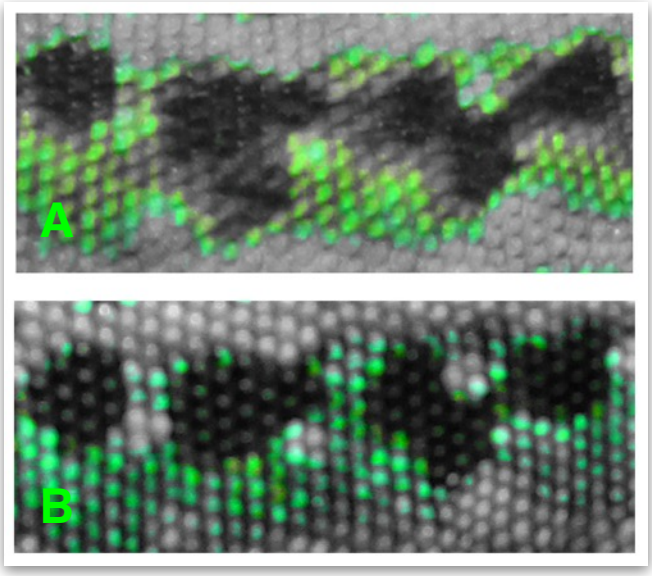


Fig 23. Exaggeration of the green channel.

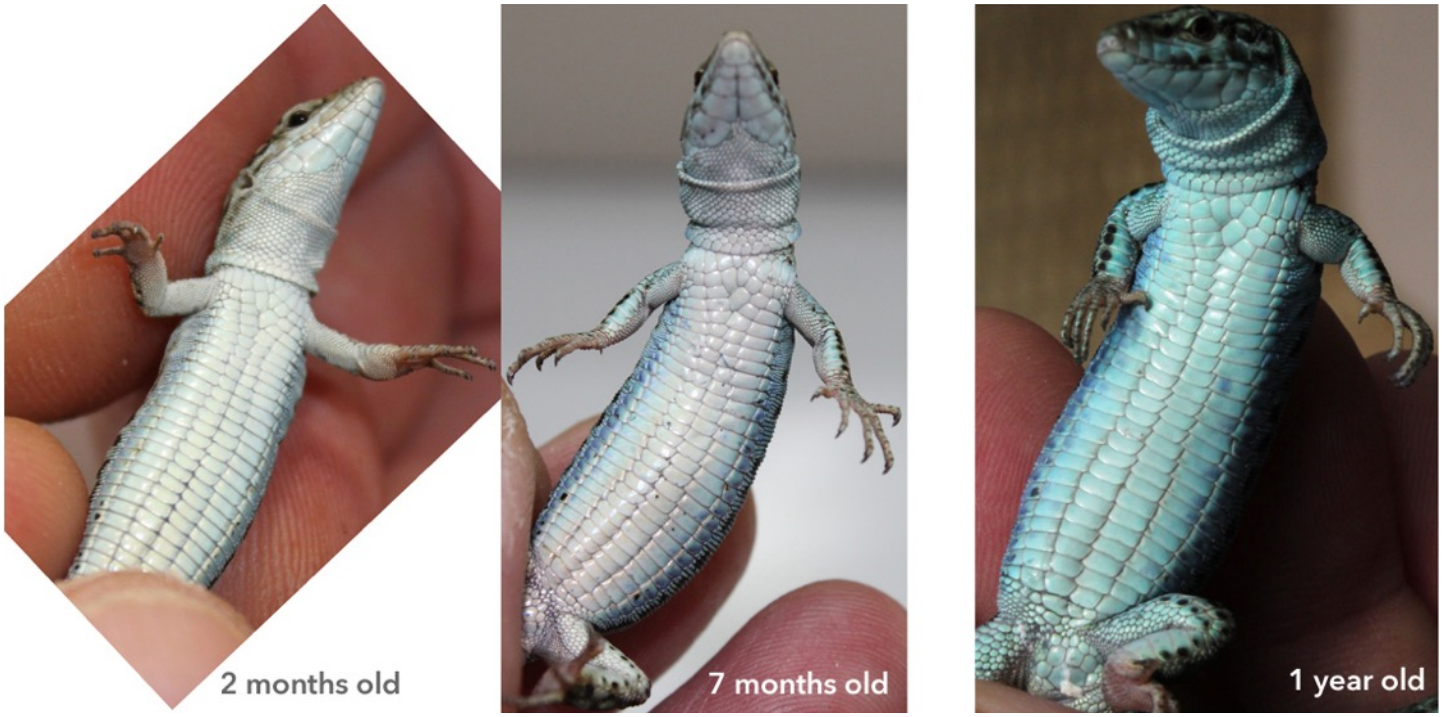


Fig 24. Ontogenetic color change in our *Podarcis pityusensis formenterae* study specimen. The "reflecting" guanine crystals change from unorganized (structural white) to organized (structural cyan).

Ventral comparison between 2 months and 1 year

When we compare the ventral side of our study specimen, we observe a gradual change in coloration from whitish, at the age of 2 months, to cyan, at the age of 1 year. This suggests an ontogenetic change in the organization of the photonic crystals, from unorganized (structural white) towards organized (structural cyan), as long as the situation in *Podarcis pityusensis* is similar to *Phelsuma* (Fig 25).

However, when we exaggerate the green and cyan channel with +75 (Fig 26 and 27), we can observe some green coloration in the youngest version of our lizard, which clearly is replaced by a cyan coloration when getting older. It seems that a little organization of the photonic crystals already exists at the age of 2 months, and that this organization of the photonic crystals becomes more uniform and complete.

When this situation could be explained proven in a forthcoming study, the mechanism of this rearrangement within the iridophores might be another topic for future research.

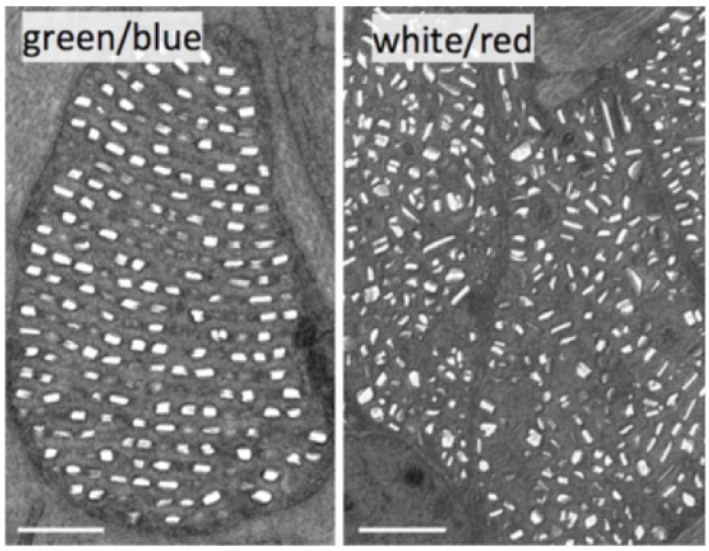


Fig 25. Electron microscope image of structural green/blue and structural white iridophores in *Phelsuma*. (From SAENKO et al. 2013).

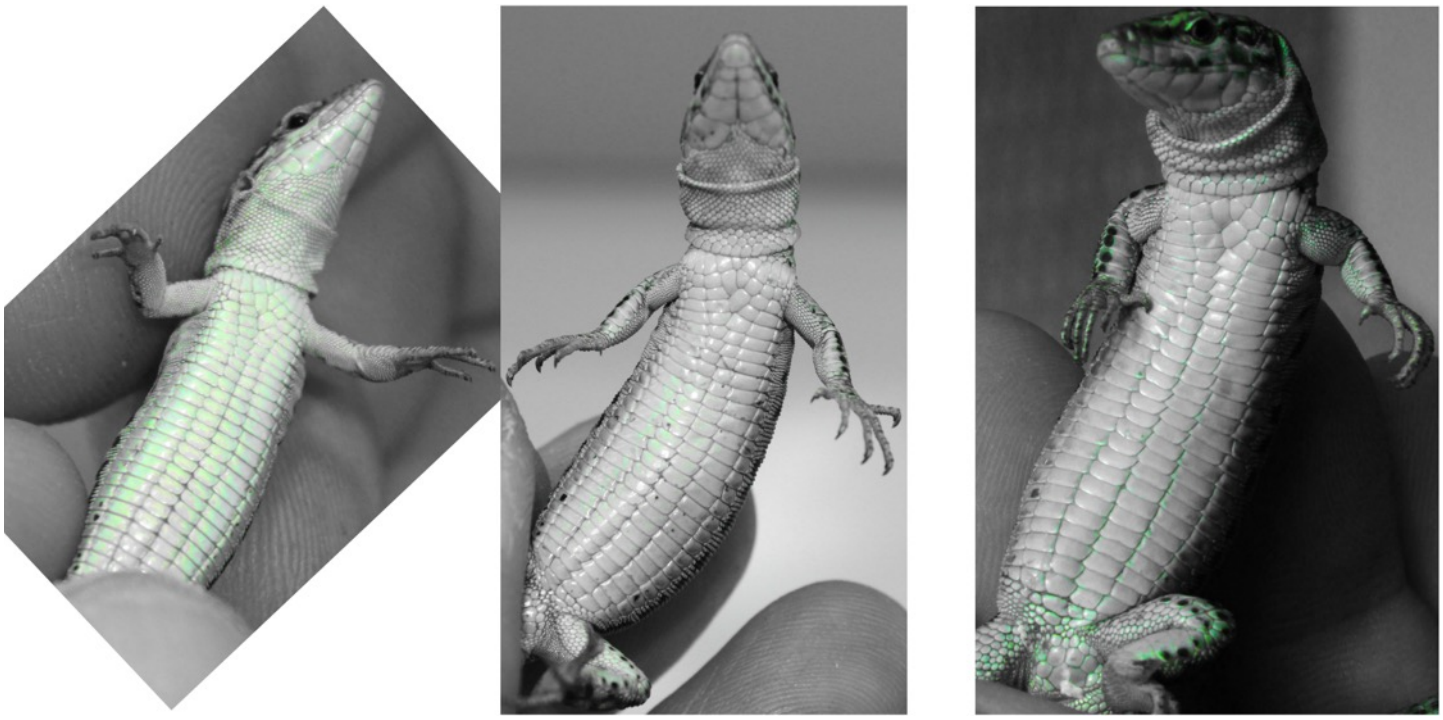


Fig 26. Exaggeration of the green channel by 75%. Note the faint green parts in the youngest stage.

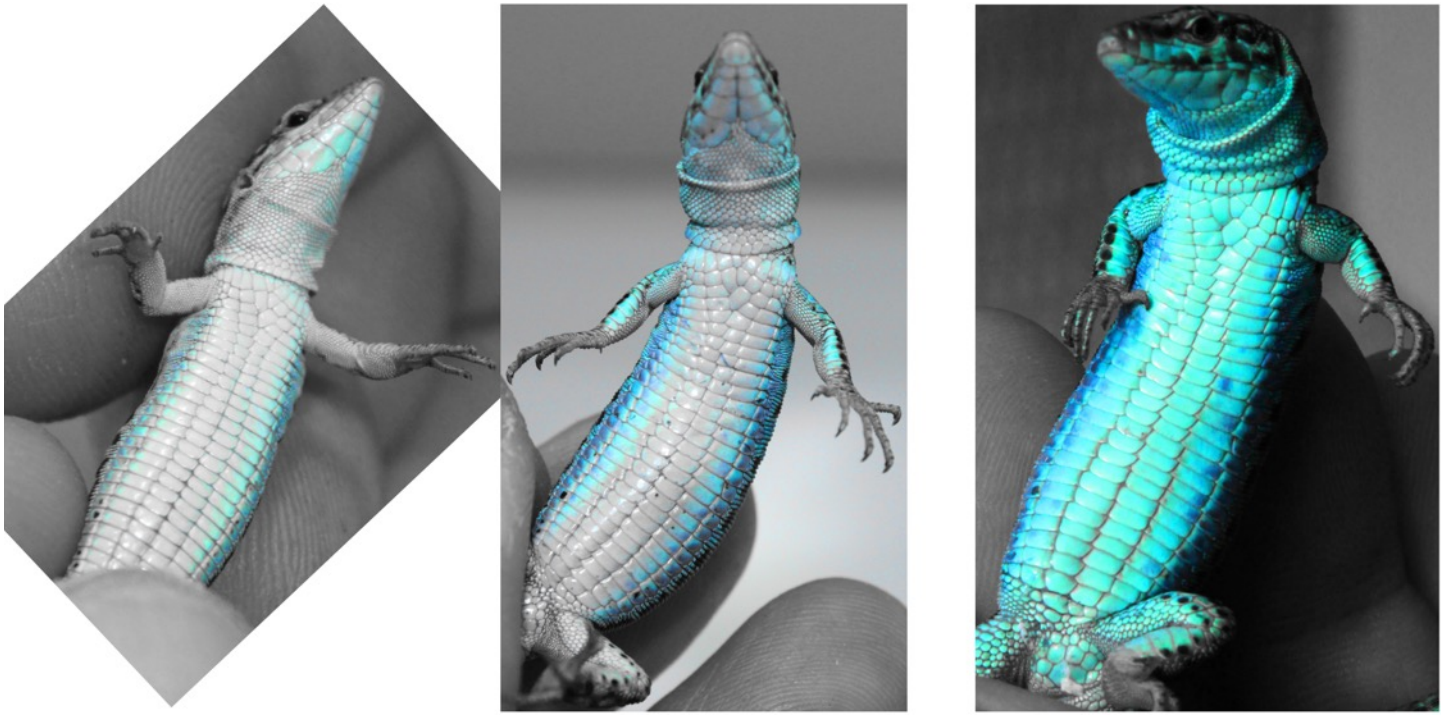


Fig 27. Exaggeration of the cyan channel by 75%. During aging, a clear shift towards uniform cyan can be observed.

Fig 28. The female *Podarcis pityusensis formenterae* study specimen, almost 3 years old (2015). Note the absence of changes in coloration, compared to the 1 year old situation.

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