Scientific Note: Blending with the background: very unusual background-matching behavior of a caterpillar of *Ariadne merione* (Nymphalidae) in captivity

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Abstract: Background matching or camouflaging is a very common yet interesting behavior shown by different taxonomic groups. Background matching is a form of crypsis, which is an adaptive strategy against predation. Camouflaging through background mimicking is mostly reported in adults but recorded less frequently in early stages of development. In Lepidoptera, this behavior in the larval stage is extremely rare, observed in only a few moth species. There are some reported instances of larval polyphenism but there is no report of background mimicking in butterfly larvae. In this note, we are reporting a very interesting and probably the first instance of background matching of a caterpillar of *Ariadne merione* butterfly (Lepidoptera: Nymphalidae) in captive rearing where the caterpillar mimicked the color of the lid of the rearing box.

Key words: Background mimicking; background selection; camouflaging; chromatophore; structural coloration.

Camouflaging or background matching is an anti-predatory adaptive strategy displayed by different taxonomic groups, from arthropods to reptiles (Wallace, 1879; Wallace, 1889). Camouflage can be achieved in two ways. In one group with fixed phenotypic variation, background matching is achieved by ‘background selection’ or ‘background choice’, whereas in the other group matching is mediated by ‘background mimicking’ or ‘plastic color change’. In lepidopterans, the most observed mode of camouflaging is ‘background selection’ (Sargent, 1966; Kang *et al.,* 2015), a behavioral response observed in both early stages and adults whereby individuals move to rest upon a background that better matches their own body color. In nature, many cryptic species of butterfly, irrespective of whether they are monomorphic or polymorphic (genetically defined) or polyphenic (environmental factors induced), blend with a fixed range of background depending on their wing color. For example, the Dry Season Forms (DSF) of some Satyrinae butterflies provide effective camouflage on dry leaf litter (Kunte, 2000). Camouflaging through background selection is observed in early stages as well. In some species of butterfly the larval period includes two or more distinct genetically defined phenotypes, each of which has different camouflaging ability. For example, the early larval instars of some *Papilio* Linnaeus, 1758 species are known as the ‘bird dropping stage’, as this stage strongly resembles bird droppings, whereas the later instars are entirely green in color, which helps them to camouflage on the green leaves of their host plants (Futahashi & Fujiwara, 2008a,b; Jin *et al.*, 2019). Many species of butterfly show pupal color plasticity, where the color of the pupa strongly blends with the color of the pupation substrate (Fujiwara & Nishikawa, 2016; Mayekar & Kodandaramaiah, 2017). In the satyrine *Mycalesis mineus* (Linnaeus, 1758), under high relative humidity, a higher proportion of green pupae are formed on the underside of leaves, and under low relative humidity a higher proportion of brown pupae are produced in the soil (Mayekar & Kodandaramaiah, 2017).

However, despite there being a considerable number of examples of ‘background choice’, the occurrence of ‘background mimicking’ or pigment-based ‘plastic color change’ is extremely rare in early stages of Lepidoptera. Some of these instances include color plasticity in the larval stage of the geometrid twig-mimicking Peppered Moth, *Biston betularia* (Linnaeus, 1758) (Eacock *et al.,* 2017) and the pupal stage of the nymphaline butterfly Small Tortoiseshell, *Aglais urticae* (Linnaeus, 1758) (Poulton, 1890). However, this type of color change can be rapid or slow, and the degree of color matching also varies from species to species. Rapid change in color has been observed and thoroughly studied in higher taxonomic groups such as cephalopods, fish, amphibians and reptiles (Hanlon *et al.,* 2009; Buresch *et al.,* 2011; Fulgione *et al.,* 2014; Ramirez and Oakley, 2015; Allen *et al.,* 2015; Kingston *et al.,* 2015; Schweikert *et al.,* 2018), whereas slow change in color is mostly observed in arthropods (Bückmann, 1977; Insausti & Casas, 2008). As crypsis is an adaptation against detection by visually hunting predators like birds (Merilaita *et al.,* 2001), generally this pigment-based color change is mostly associated with sensing background either through visual cues, as seen in higher taxa such as most amphibians and reptiles (Mathger & Hanlon, 2007; Vroonen *et al.,* 2012; Kang *et al.,* 2016), or through extraocular reception, as observed mostly in arthropods

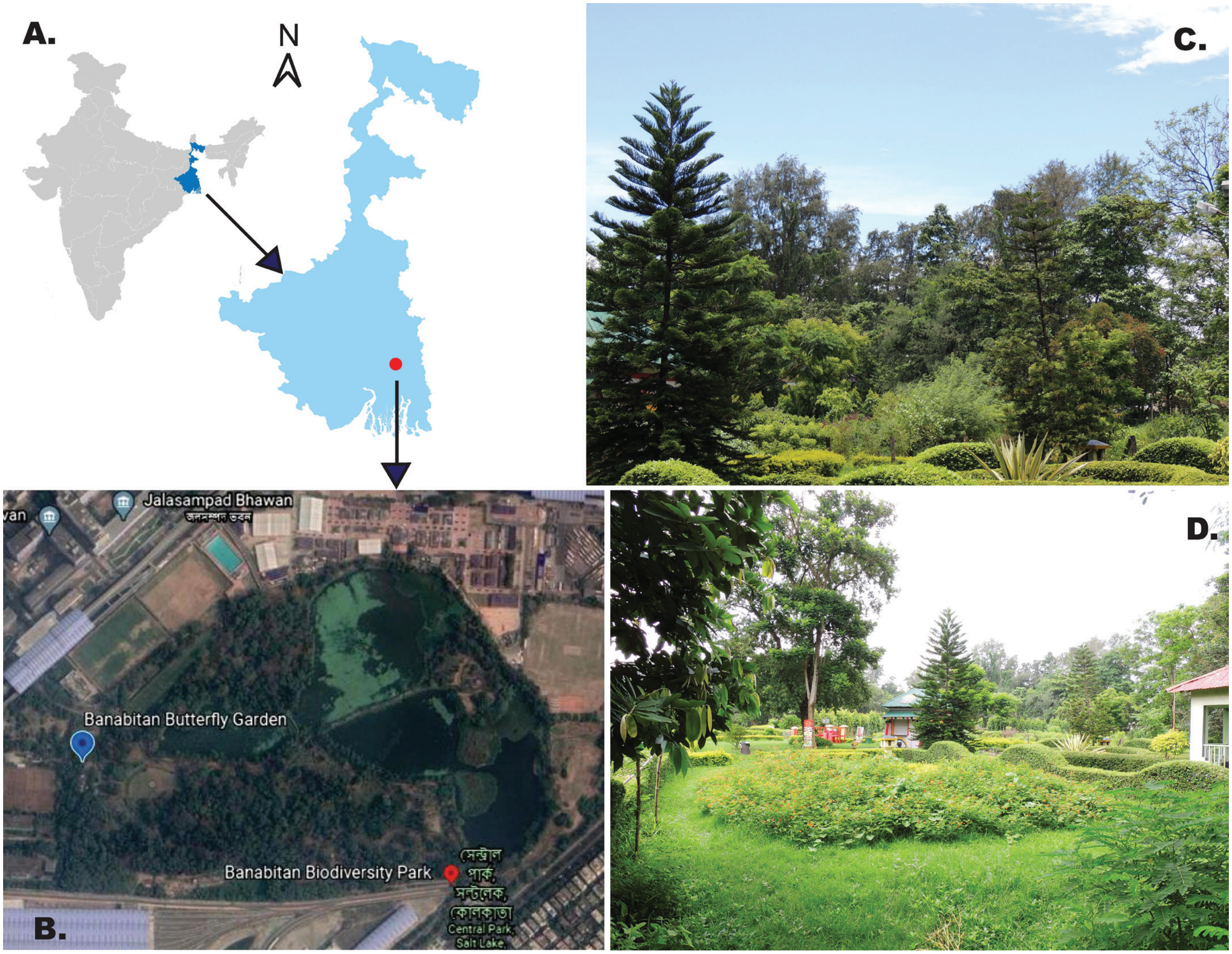

Figure 1: Location and habitat of study area. A. Location of Banabitan Biodiversity Park, B. Satellite image of Banabitan Biodiversity Park along with Banabitan Butterfly Garden (Blue pointer) (Source: Google Earth), C. and D. Habitat of Banabitan Butterfly Garden.

(Eacock *et al.,* 2019). Still, we do not know many aspects of camouflaging in many systems, and the cascading effects behind them. It is also unknown whether or not the degree of camouflaging varies in an artificial environment compared with the natural environment.

In this scientific note we capitalize on an opportunistic observation to provide the first report of 'background matching' in captive caterpillars of *Ariadne merione* (Cramer, [1777]) (Lepidoptera: Nymphalidae), a species which generally prefers background selection in its larval and pupal stage in the wild. *Ariadne merione* is a widespread tropical Asian butterfly, occurring in India in the Western Ghats, central India, north and north-east India (Saji *et al.,* 2020). The study reported here was carried out at Banabitan Butterfly Garden (22°35'12''N, 88°24'53''E), situated at Banabitan Biodiversity Park in Kolkata, West Bengal, India (Figure 1). We collected a single batch of 4 freshly laid eggs of *Ariadne merione* on 24th February, 2014 from Banabitan Biodiversity Park. The eggs were laid on the underside of a leaf of *Ricinus communis* L. (Euphorbiaceae). We placed the collected eggs on a glass petridish and all of the collected eggs hatched on 28th February, 2014. After the eggs hatched, we transferred the larvae into a plastic rearing box with a blue lid (Figure 2C). We raised the caterpillars inside the rearing box under a controlled temperature (23°C-25°C). We provided them with fresh leaves of *Ricinus communis* and cleaned the frass as well as the remaining uneaten portion of the leaves on a daily basis, to maintain healthy and hygienic conditions. The caterpillars were primarily green in color up to the 3rd instar. However, on entering the 4th instar, one of the 4 caterpillars suddenly appeared as blue, while the rest remained green. We observed this on 9th March, 2014. Surprisingly, the shade of blue on the caterpillar's body was the same as the color of the lid of the larval rearing box, strongly suggesting a background matching ability in the caterpillar. On observing this unusual color form, we photographed the caterpillar with a NIKON COOLPIX P510 camera (Figure 2).

Close observation under magnifying glass of the above-mentioned caterpillar in the late 4th instar revealed that the entire exoskeleton took on the blue color. The thoracic legs and abdominal prolegs were also blue. The color of the two rows of lateral spines also turned blue (instead of green), whereas the dorsal spines remained the same color as in normally colored

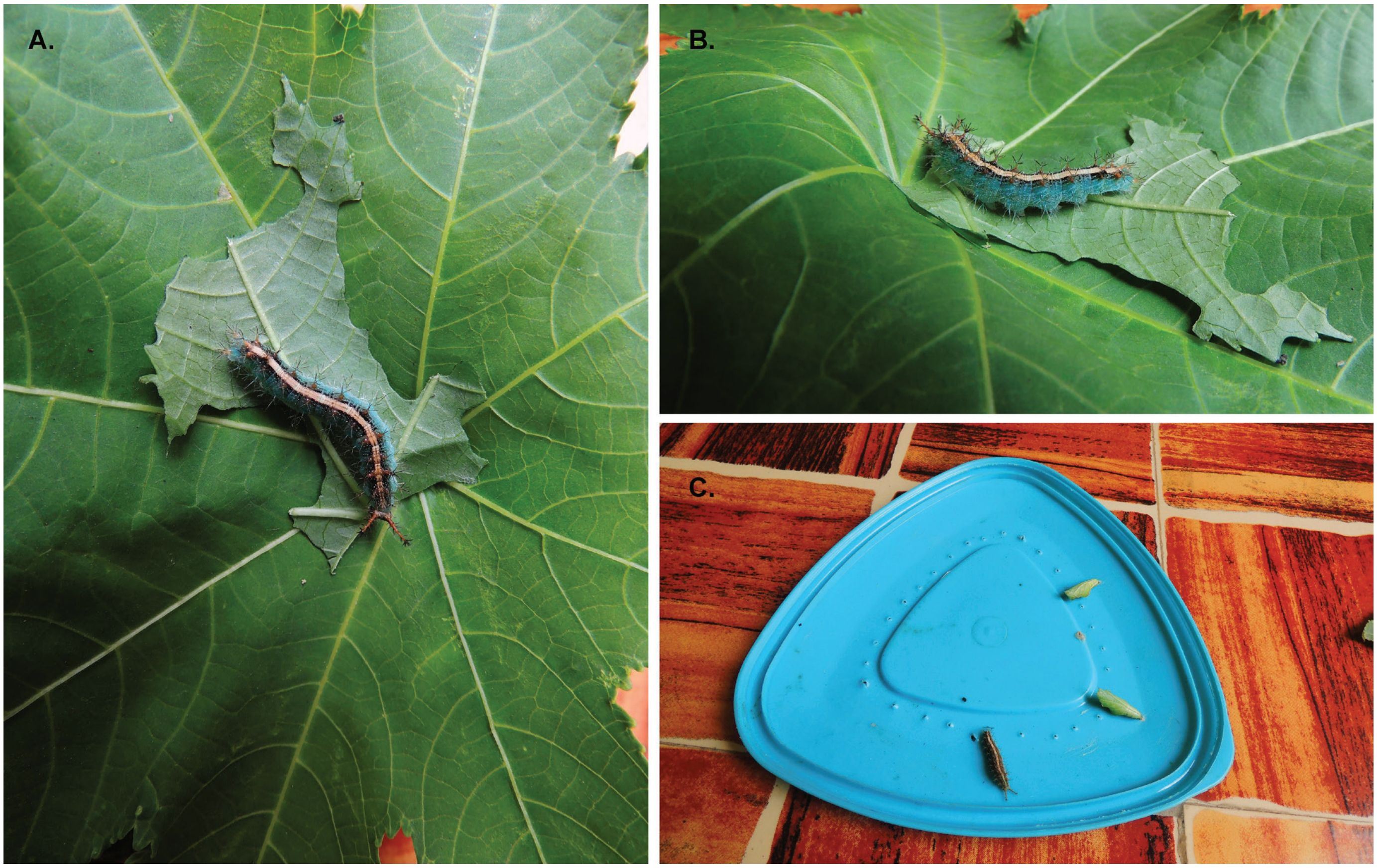

Figure 2: Observation of the background mimicking behavior in caterpillar of *Ariadne merione.* A. Camouflaged caterpillar from dorsal view, B. Camouflaged caterpillar from lateral view, C. Lid of the container in which the camouflaged caterpillar was reared.

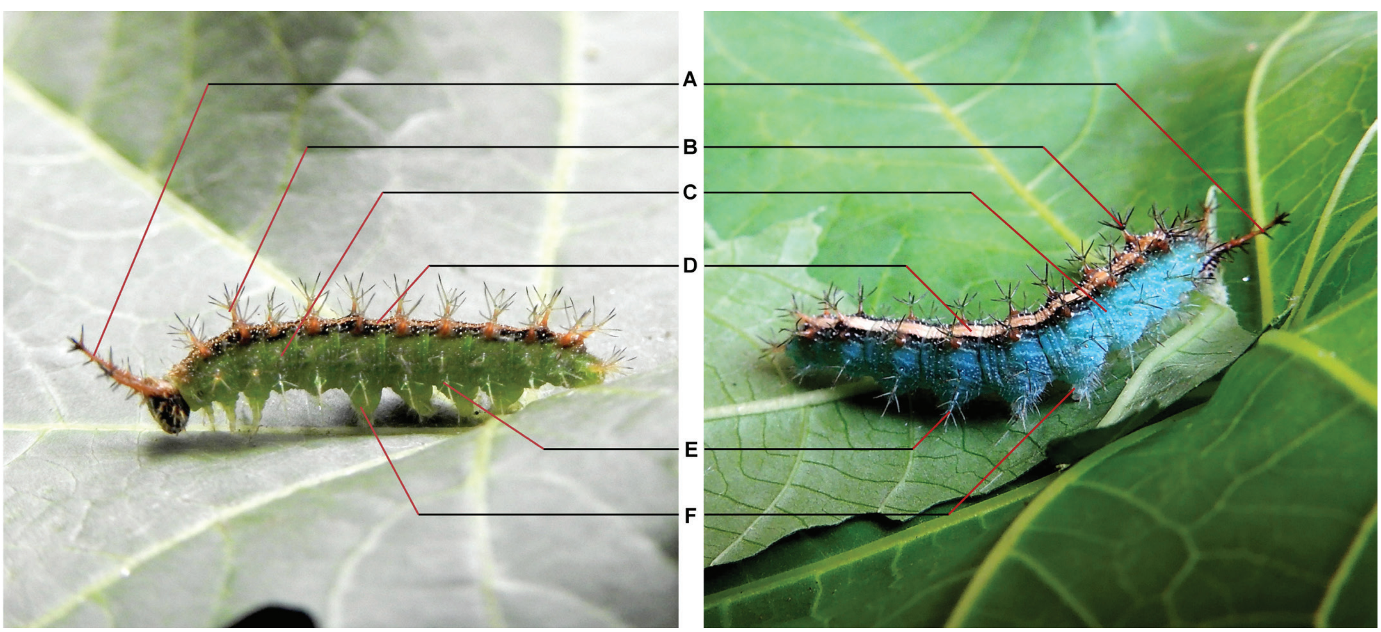

Figure 3: Comparative study of external morphology between a normal colored caterpillar (left) and the camouflaged caterpillar (right) of *Ariadne merione*. A. Horn, B. Dorsal spine, C. Exoskeleton (skin), D. Dorsal stripe, E. Lateral spine, F. Abdominal proleg.

caterpillars (spines with a brown base, brown shaft and black branched tips). The lines traversing both sides of the caterpillar were also blue instead of green. The color of the longitudinal dorsal stripe of the caterpillar was the same as in other caterpillars (a creamy yellow band flanked by black edges). The color of the head (blackish brown with white markings) and one pair of horns (reddish brown with black tip) were also similar to those of normal caterpillars (Figure 3) (Atluri *et al.,* 2010).

We monitored the caterpillar throughout its larval period. A regular follow-up on the growth of the caterpillar revealed that

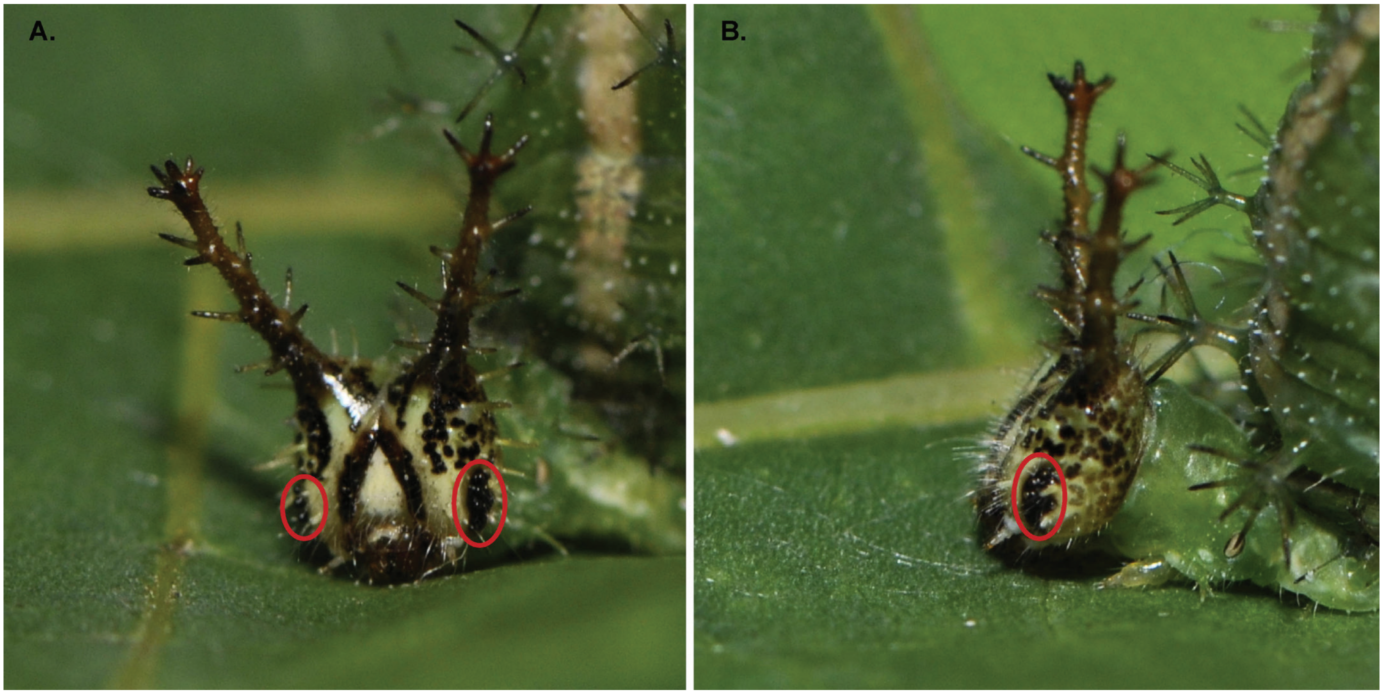

Figure 4: Ocelli (simple eye) of a caterpillar of *Ariadne merione*. A. Frontal view, B. Lateral view.

it retained its blue coloration until the final instar, but before pupation the pre-pupal stage again acquired the usual brown color of the exoskeleton. The caterpillar pupated on 14^{th} March, 2014 and the color of the pupa was also similar to a normally colored pupa (green). The adult emerged on 21^{st} March as a normally colored individual (dorsally brownish ochraceous, ventrally dark brown) and was released into the wild. We also recorded the duration of the early life history stages and found a larval period of 13 days and pupal period of 7 days for this particular individual, which is similar to the typical early stage duration of *A. merione* (personal observation).

In Lepidoptera, larval color plasticity is generally associated with different environmental factors such as temperature and photoperiod, resting substrate, larval diet and many more (Greene, 1989, 1996; Hazel, 2002; Eacock *et al.,* 2017). However, background matching in lepidopteran larvae (such as in *Biston betularia* and the sphingids *Laothoe populi* (Linnaeus, 1758), Poplar Hawkmoth and *Smerinthus ocellatus* (Linnaeus, 1758), Eyed Hawkmoth) is primarily mediated through sensing background color, more specifically background reflectance and the wavelength of light coming from the surroundings (Grayson & Edmunds, 1989; Noor *et al.,* 2008). In Poplar Hawkmoth and Eyed Hawkmoth, color plasticity in final instar caterpillars is aided through perceiving the reflectance quality of the substrate color on which they rest (in the wild it is generally the leaves of their host plants) by means of 'ocelli' (Grayson & Edmunds, 1989; Edmunds & Grayson, 1991), whereas in Peppered Moth some additional extraocular receptors play a significant role in sensing the wavelength of background color (Eacock *et al.,* 2019). Though *Ariadne merione* caterpillars also possess simple eyes or ocelli (Figure 4), like other lepidopterans, it was not clear from our single observation whether this color change was associated with any visual cues sensed by the ocelli. This is because we didn't get the opportunity to transfer another blue caterpillar into a new rearing box with a different lid color but similar rearing environment and check whether it again matched the new background color. We also do not know what caused just this larva to turn blue and not the others in the container. In fact, in subsequent years we raised several thousand *A. merione* caterpillars in the same blue-lidded container, as well as in other containers with different lid colors (yellow, pink, red and opaque white), but didn't observe any repetition of the background matching behavior. Furthermore, we did not obtain any clues as to whether that particular caterpillar would still change its color under natural conditions, but we have not encountered any such unusual behavior in *A. merione* larvae in nature.

Coloration in animals can be achieved in three ways: through pigmentation, structural coloration and bioluminescence. The presence of bright blue pigment is extremely rare in the animal kingdom. Until now, only five Neotropical nymphalid butterfly species in the genus *Nessaea* have been reported to possess 'pterobilin', a brilliant blue wing color pigment (Choussy & Barbier, 1973). This rarity of occurrence suggests that, in our case, the blue coloration of *A. merione* caterpillar was probably not caused by any blue pigment. Moreover, we observed the background matching in the *A. merione* caterpillar in the 4^{th} instar, but there was no sign of such a plastic color change until the 3^{rd} instar, which suggests that the modulation of color in the newly developed exoskeleton took place during the late 3^{rd} instar stage prior to the 3^{rd} ecdysis. These observations indicate a fast color change or a phenotypic switch, unlike other color-changing moth caterpillars which mostly show slow and gradual color changing in between two molts (Grayson & Edmunds, 1989; Noor *et al.,* 2008; Eacock *et al.,* 2017). This rapid color switch hints that the blue color of the *A. merione* caterpillar could result from a structural color change, which can happen very quickly (within a few seconds to a few minutes), through the aid of chromatophores and iridophores, as is the case in cephalopods such as octopuses, squid and cuttlefish (Mäthger *et al.,* 2009; Gilmore *et al.,* 2016). In some arthropod systems rapid 'physiological color change' is often mediated through chromatophore movement or rearrangement of pigment granules and structural reflective plates within chromatophores (Umbers *et al.,* 2014). In lepidopteran larvae, alteration in chromatophore content has also been reported to play a crucial role in gradual color change (Noor *et al.,* 2008), which additionally supports the possibility of involvement of chromatophore activity in the striking blue coloration of the *A. merione* caterpillar, although probably in a different way. However, more study of the structure of the larval stages of *A. merione* is required to clarify this scenario.

The adaptive and evolutionary significance of camouflaging has been studied in different systems. Slow or rapid background matching is a common and effective adaptive concealing strategy to avoid predation. Interestingly, the 1st and 2nd instar caterpillars of *Ariadne merione* prefer to settle on the underside of the leaves of *Ricinus communis*, and thus during this stage they face much less predation risk from more highly visually hunting predators such as birds, but not to insect predators, to which they may be even more vulnerable. But, as soon as the caterpillars grow larger, they position themselves on the upperside of leaves, until pupation. This behavior suggests that the later instars might be more vulnerable to avian predators due to larger body size and position on the leaves. This increased predation pressure might play a crucial role in influencing our observation of a plastic color change in the 3rd instar caterpillar which was subsequently implemented in 4th instar. More detailed study is clearly needed to test this hypothesis, since there are otherwise no records of color matching in early stages of *A. merione* in the wild.

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