

# Postembryonic development of the troglobitic planthopper species *Valenciolenda fadaforesta* Hoch & Sendra, 2021 (Hemiptera, Fulgoromorpha, Kinnaridae), with a key to nymphal instars

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## Abstract

All nymphal instars of the recently discovered troglobitic planthopper species *Valenciolenda fadaforesta* Hoch & Sendra, 2021 are described. This represents the first documentation of the complete postembryonic development of any species in the family Kinnaridae. Characters of the external morphology are described and illustrated, and a key to the instars are provided to facilitate discrimination among the different nymphal stages. While *V. fadaforesta* nymphs share certain synapomorphies with other Fulgoromorpha (except the Tettigometridae), e.g., the cog-wheel structures of the metatrochanters, other characters may be correlated with the subterranean way of life of the species, and thus be autapomorphic, such as the absence of compound eyes in all instars.

**Keywords**

Caves, Iberian Peninsula, morphology, taxonomy, troglobite

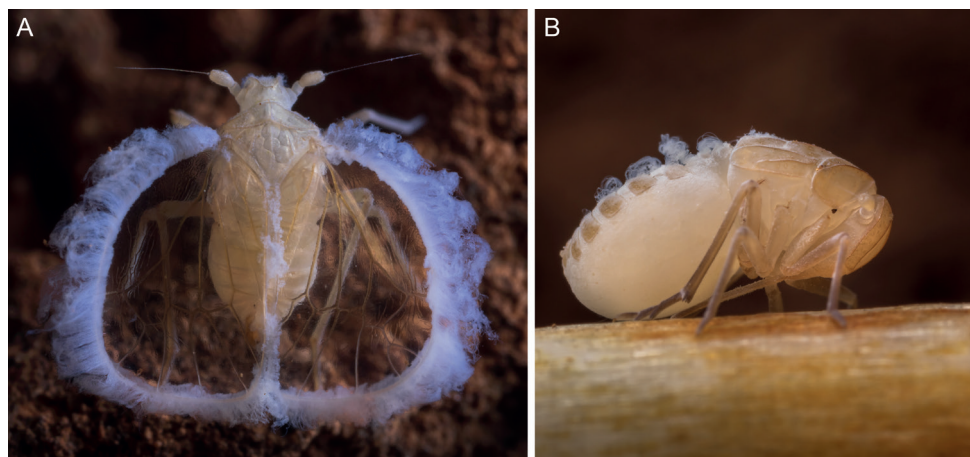
**Introduction**

Fulgoromorpha is a large subgroup of the Hemiptera comprising at least 14,000 species in 21 extant and 15 extinct families (Bourgoin 2022). Despite their worldwide distribution (Bourgoin 2022), ecological diversity and economic importance (Denno and Perfect 1994), and many aspects of their biology are scarcely known. While postembryonic development has been studied in more detail in select Fulgoromorpha taxa (see e.g., Yang and Yeh 1994, and references therein, Emeljanov 1996; Asche 2015), information on nymphal morphology remains incomplete or lacking for most species. The only comprehensive treatments of Fulgoromorpha nymphs to date, including representatives of most taxa in family rank to date has been provided by Yang and Yeh (1994) and Emeljanov (2001). From their observations Yang and Yeh (1994) derived a compilation of characters considered to be distinctive of the 5<sup>th</sup> instar of Fulgoromorpha nymphs, and Emeljanov provided a hypothetical ground plan of certain aspects of nymphal morphology of Fulgoromorpha. One of the taxa which were neither covered by Yang and Yeh (1994) nor Emeljanov (2001) are the Kinnaridae. This family currently consists of 116 described species across 25 genera from the Palearctic, East Africa (partim), Arabian Peninsula and Oriental regions, as well as from the Nearctic and the Neotropics (Bourgoin 2022).

The nymphs of Kinnaridae have until recently been entirely unknown. This is most likely due to the lack of material, the reason for which may lie in their cryptic way of life. These apparently live in or close to the soil and/or subterranean crevices presumably feeding on roots (Fennah 1948, 1980). The nymphs of epigeic species supposedly leave the nymphal habitat before or after the adult molt and feed and reproduce above ground, while adults of troglobitic species complete their life cycle underground. Cave-dwelling species of Kinnaridae are documented from Mexico (Fennah 1973), Jamaica (Fennah 1980), Brazil (Hoch and Ferreira 2013, 2016) and Spain (Hoch et al. 2021).

The first description of a kinnarid nymph (instar V) was given by Hoch et al. (2021) for the troglobitic *Valenciolenda fadaforesta* Hoch & Sendra, 2021 from Spain. Described from a cave in eastern karstic massif in the east of Iberian Mountain Range, in Valencia, Spain (the type locality), adults and nymphs were found to co-occur in the same habitat, thus offering the opportunity to obtain and study nymphs of all instars (Fig. 1).

Here we provide the first descriptions of the external morphology of instars I–IV of a representative of the Kinnaridae and summarize the results of Hoch et al. (2021). Our observations contribute to the knowledge of morphological alteration during nymphal development and help to complete the ground pattern of nymphal instars in Fulgoromorpha.



**Figure 1.** *Valenciolenda fadaforesta*, habitus of adult male (left) and instar nymph V (right). Body length of adult: 3.6 mm; body length of nymph: 2.9 mm. (Photo courtesy by Roberto García-Roa, [www.robertogarcia-roa.com](http://www.robertogarcia-roa.com)).

## Methods

### Sampling

Immatures of *Valenciolenda fadaforesta* were located visually and by direct intuitive searching (DIS, sensu Wynne et al. 2019) within an undefined search radius, yet with special emphasis around root patches, in the deep cave zone at the type locality of the species: Murciélagos Cave, in Les Rodanes Municipal Natural Park which is located in Villamarchante, Valencia, Spain. Specimens were collected by hand during three visits to the cave, and immediately transferred to vials containing 70% ethanol (for dates and number of specimens see Table 1). As the present study focuses on the qualitative description of the nymphal instars of *V. fadaforesta*, we deliberately refrained from frequent visits and overcollecting because the cave in question is only one out of two localities where this species occurs. Since we do not have any knowledge about population size and life history of the species, we aimed at minimal impact to the environment and to the species. Measurements of body length were taken from freshly collected specimens using a Leica DMLS phase-contrast optical microscope with an ocular micrometre. For further examination specimens were washed with distilled water and then mounted on glass slides with Marc André II solution.

**Table 1.** Collection dates and number of nymphs per instar stage for each of the five instar stages, Murciélagos Cave, Les Rodanes Municipal Natural Park, Valencia, Spain.

Date	Instar I	Instar II	Instar III	Instar IV	Instar V	
11.09.2020	0	1	6	8	2	
18.10.2020	1	2	2	5	5	
28.11.2020	4	6	5	1	1	
Total	n = 5	n = 9	n = 13	n = 14	n = 8	n = 49

## Morphological analysis

For morphological analysis and drawings, a Leica MZAPO stereoscopic microscope with *camera lucida* attachment was used. For medium-sized and larger specimens, the right metathoracic leg was removed and mounted separately, again with Marc André II solution. For scanning electronic microscopy, specimens were critical point-dried in a Tousimis Autosamdi 815, series A, coated with palladium-gold, and examined under Hitachi S-4100 and JEOL JSM-6335F (SEM).

## Morphometric analysis

For morphometric analysis of leg proportions (length of femora and tibiae) we applied linear regression using measurements of the right metathoracic tibiae and femora from the legs mounted with Marc André II solution. We used OriginPro 8.0 software (Origin-Lab Corporation, Northampton, MA, USA) to test for suspected allometric growth.

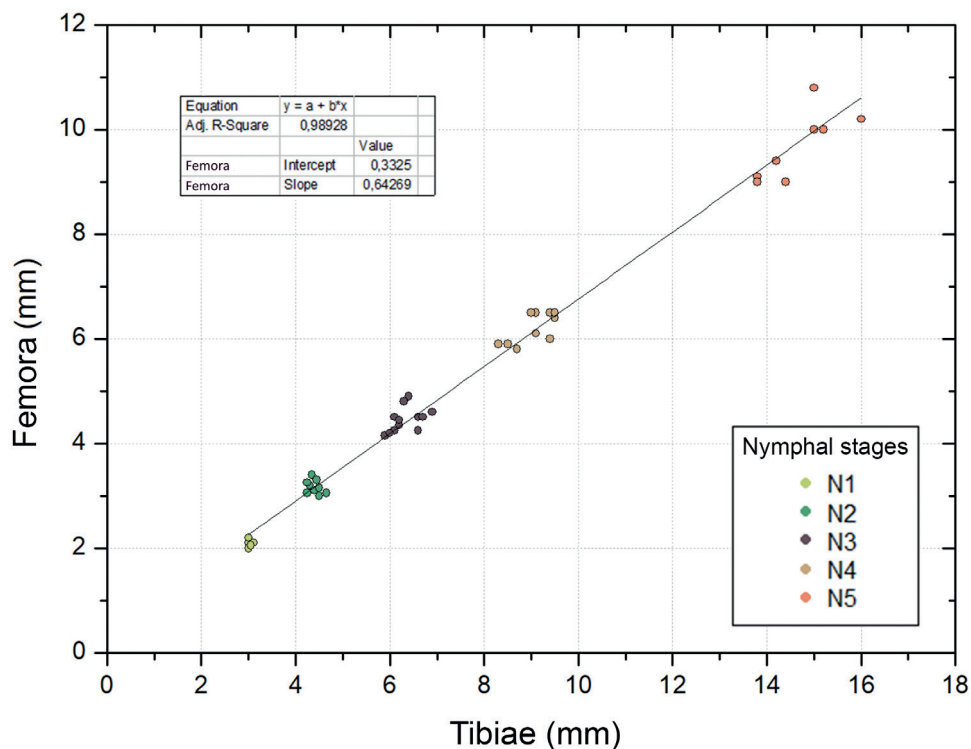
## Results

### Assigning specimens to instars

*Valenciolenda fadaforesta* has five nymphal instars which coincide with the existing records for Fulgoromorpha. As overall body size varied widely among specimens studied and given the small sample size, it was not possible to discern nymphal instars based on this character alone. However, the linear regression analysis (length of femora/tibiae of the hind legs) revealed that our data points fell into 5 “groups” (Fig. 2). Upon closer morphological examination, we found that these groups coincided with the nymphal stages in other Fulgoromorpha as described by Emeljanov (2001). Separating the instars with certainty is thus possible on the basis of morphological characters (e.g., presence or absence of wing pads, shape and relative size of wing pads, etc.)

### General diagnosis of nymphal stages

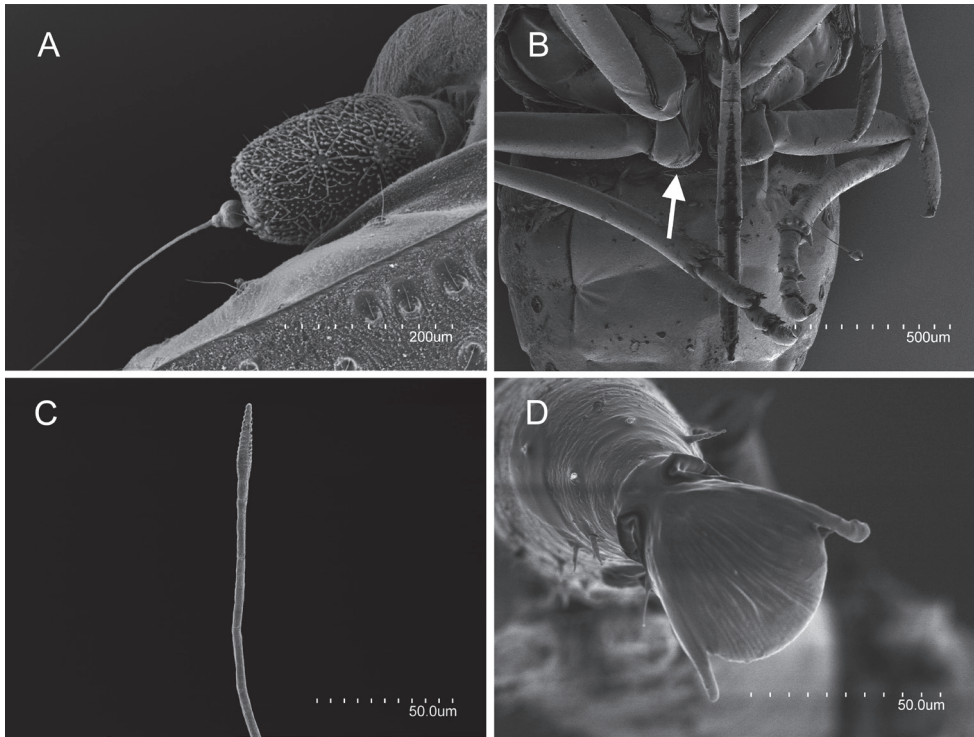
*Valenciolenda fadaforesta* nymphs are generally small, with body length, i.e. the distance between the anterior margin of the vertex to the caudal margin of the abdomen, ranging from 0.8 mm (1<sup>st</sup> instar, N1) to 3.2 mm (5<sup>th</sup> instar, N5). Bodily pigmentation is largely reduced, the integument is predominantly white; compound eyes and ocelli are absent, tegulae and wings are vestigial. The body is dorsoventrally depressed, longer than wide, with maximum width in the second thoracic segment at the base of the wing pads, maintaining approximate proportions in all nymphal stages. Frons, thoracic nota and abdominal segments IV–VIII covered by a variable number of sensory pit organs. The median tergite of the metathoracic segment (metathoracic postnotum) features a variable number of wax-secreting pores.



**Figure 2.** Regression analysis of metathoracic leg length in the nymphal instars of *Valenciolenda fadaforesta*.

**Colouration:** Vertex, frons, thoracic and abdominal nota, tibiae and tarsi yellowish-white; ventral regions of thorax and abdomen, coxae and femurs white; head and thorax carinae, rostrum and lateral teeth of metathoracic tibiae yellowish-brown.

**Head:** Vertex short, significantly wider than long medially. Frons narrow, ca. 1.45 times longer than maximally wide, with maximum width at the level of the antennae, devoid of median carina and provided with two submedial carinae that delimit a smooth area devoid of sensory pits. Frons separated from vertex by distinctly ridged margins. Between submedial carinae and lateral margins three rows of sensory pits that cover the section up to the frontoclypeal suture. Frontoclypeal suture almost straight, slightly curved in the medial section. Postclypeus and anteclypeus smooth, devoid of carinae. Elongated rostrum, well surpassing caudal margin of the metathoracic coxae by more than half its total length. Compound eyes absent. Antennae with 17 antennomers, scape short, ring-like, in some specimens not clearly visible, retracted into head capsule; pedicel subglobular, covered by plate organs (their number varying among instars) (Fig. 3); arista long and thin, composed by the 15 remaining antennomers. Third antennomer thickened, with a singular plate organ. Last antennomer slightly thickened, club-shaped (Fig. 3).



**Figure 3.** *Valenciolenda fadaforesta* nymphal structures (SEM) **A** V instar, base of antenna: scape, pedicel and antennomere III (dorsal view) **B** IV instar, cog-wheel structure at the trochanters of the hind legs, characteristic for all Fulgoromorpha (except the Tettigometridae) **C** III instar, distal antennomers **D** V instar, tip of the pretarsus, showing the claws and the arolia.

**Thorax:** Pronotum short, tricarinate, ca. 2.3 times wider than head at level below antennae. Median carinae dividing the pronotum into a small anterodorsal region, barely visible in dorsal view, and a triangular-shaped dorsal region endowed with a variable number of sensory pits depending on the nymphal stage. Mesonotum tricarinate and slightly wider than medially long. Median carinae separates mesothoracic postnotum from mesothoracic alinotum. Postnotum and alinotum furnished with a variable number of sensory pits. Alinotum with a variable number of longitudinal carinae depending on the nymphal stage and the degree of development of the wing pads. Tegulae vestigial. Metanotum slightly wider than medially long and tricarinate. Median carinae separates metathoracic postnotum from metathoracic alinotum. Metathoracic postnotum endowed with a variable number of wax-secreting pores depending on the nymphal stage. Metathoracic alinotum with an inconspicuous expansion on margins, vestige of the hind wing, and with a variable number of sensory pits.

**Legs:** Trochanters of the hind legs medially with "cog-wheel"-like structure (as in nymphs of other Fulgoromorpha families, except Tettigometridae (Asche 1988)). This structure apparently facilitates the synchronization of hind legs during jumping (Burrows and Sutton 2013) (Fig. 3). Metathoracic coxae with meracanthus. Metathoracic tibiae laterally unarmed, with 7–8 slender teeth, arranged in a slightly concave row in its most distal region. Pretarsus with claws slender, short, not exceeding the arolium (Fig. 3). The proportions of meta-femur and meta-tibia undergo gradual modification in the course of consecutive molts: tibial length increases more strongly than femoral length, i.e. each instar display absolutely and relatively longer hind tibiae than the previous one (Fig. 2).

**Abdomen:** Ovoid, consisting of 9 segments, about as long as head and thorax together. Nota lacking carinae, mostly smooth except for small rows of tubercles on the lower margins of each segment. First two abdominal segments narrow, creating a distinct separation between thorax and abdomen. Tergites IV–VIII possess a variable number of sensory pits depending on the nymphal stage. Tergites IX–XI forming the genital complex, surrounded by tergite VIII. Dorsal unpaired process is likely the *anlage* of the anal style (segment X), framed laterally and ventrally by narrow, elongated lobes. The two short conical processes located medially at the level of the posteroventral corner of tergite IX could be interpreted as *anlagen* of either the genital styles (of the male), or of the gonocoxae VIII (of the female).

### Key nymphal instars of *Valenciolenda fadaforesta*

- 1 Wings pads on mesonotum and metanotum absent..... **2**
- Wing pads on mesonotum and metanotum present..... **3**
- 2 Antennae provided with a single plate organ. Metanotum with two sensory pits and one pore on each side. Body length 0.8–0.9 mm ..... **Nymphal instar I (N1)**
- Antennae provided with three plate organs. Metanotum with two sensory pits and three pores on each side. Body length 1.3–1.4 mm ..... **Nymphal instar II (N2)**
- 3 Metanotum with not fully developed wing pads, not reaching the distal end of the segment ..... **4**
- Metanotum with fully developed wing pads, reaching the distal end of the segment. Antennae provided with ten plate organs. Metanotum with three visible sensory pits and fifteen pores on each side. Body length 2.8–3.2 mm. .... **Nymphal instar V (N5)**
- 4 Antennae provided with six plate organs. Metanotum with five sensory pits and seven pores on each side. Body length 1.5–1.7 mm..... **Nymphal instar III (N3)**
- Antennae provided with seven sensory plate organs. Metanotum with six sensory pits and ten pores on each side. Body length 2.4–2.7 mm ..... **Nymphal instar IV (N4)**

## Description of nymphal habitus per stage

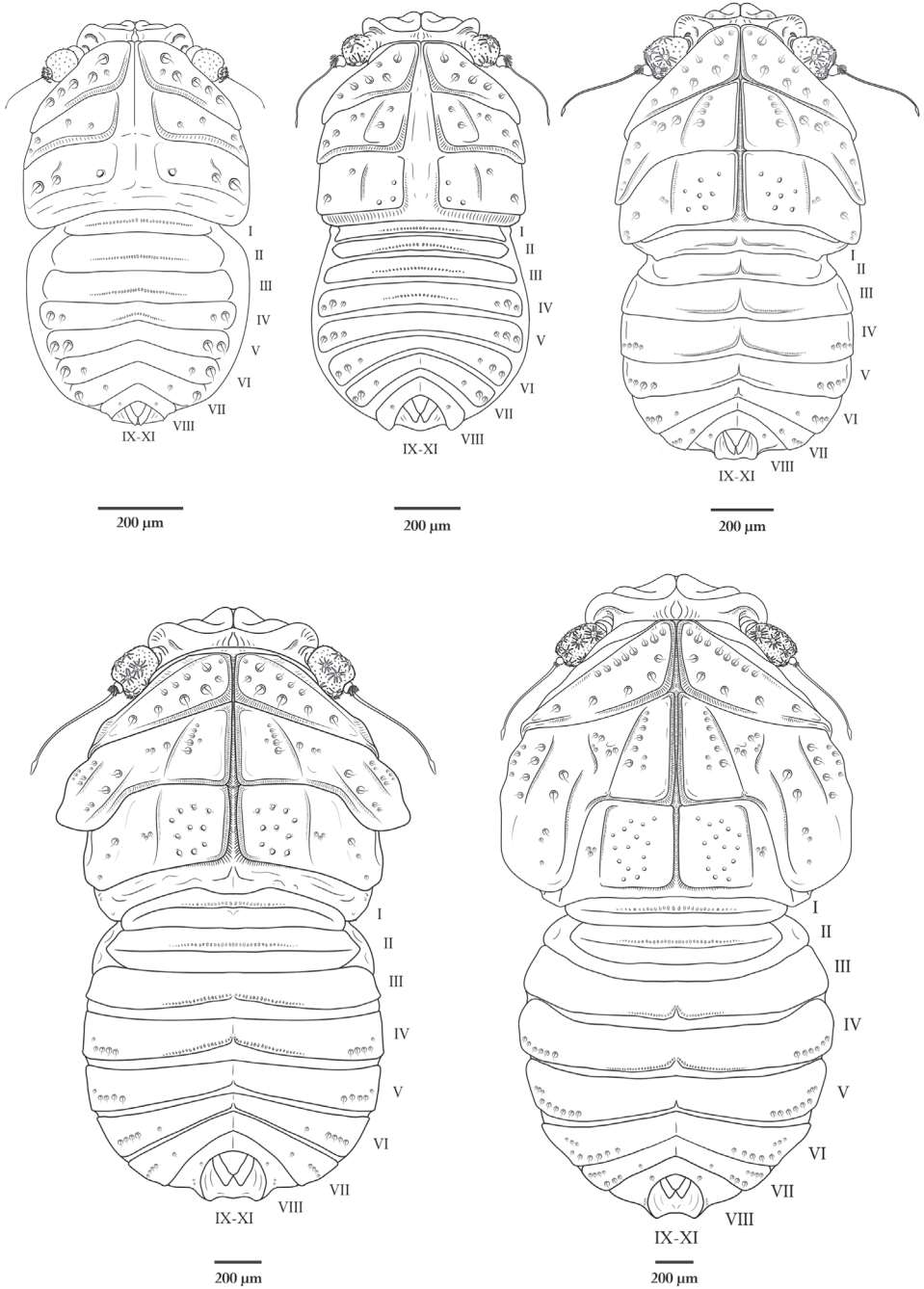
### Nymphal instar I (N1)

Small body, with a total body length between 0.8–0.9 mm (n=5). Antennae have a single plate organ located on the third antennomer and a subglobular pedicel devoid of plate organs. Tergites of the thoracic nota soft, weakly sclerotized, separated from each other by membranous area. Pronotum with 1 well-marked intermediate carina on each side of the body, separating the anterodorsal region of the pronotum, hardly visible from the dorsal perspective of the body, from the triangular dorsal region. Total absence of medial carinae in the mesonotum and metanotum that allows differentiating the tergites that make up these nota. Wing pads on meso- and metanotum absent. Sensory pits arranged in the thoracic nota as follows: pronotum with a total of 6 sensory pits on each side of the body, 3 located on the upper margin of the pronotum and 3 more central, parallel to the lower margin of the pronotum; mesonotum with 4 small sensory pits on each side, 2 located closer to the medial region of the segment and 2 in the lateral region; metanotum with a total of 2 larger sensory pits on each side, located in the lateral region of the segment. Metathoracic postnotum possesses a single pore on each side, parallel to the caudal margin of the segment. Tergites of the abdominal segments weakly sclerotized. Segments I–IV with a small row of tubercles on the lower margin, part of the cuticle sculpture. Sensory pits arranged in the abdominal segments as follows: segments IV–VII have a pair of sensory pits on both sides with each sensory pit located at the most distal ends of each segment. Segment VIII with a single small sensory pit on each side of the body. Segments IX–XI forming the genital complex (Fig. 4).

### Nymphal instar II (N2)

Small body, with a total body length between 1.3–1.4 mm (n=9). Antennae with 3 plate organs, 2 on the pedicel and 1 on the third antennomer. Tergites of the thoracic nota with a greater degree of sclerotization, still quite separated from each other by membranous area. Pronotum with 1 well-marked intermediate carina on each side of the body, separating the anterodorsal region of the pronotum, hardly visible from the dorsal perspective of the organism, from the triangular dorsal region. Meso- and metanotum with 2 medial carinae on each side of the body dividing the segments in postnotum and alinotum. Wing pads on meso- and metanotum absent. Sensory pits arranged in the thoracic nota as follows: pronotum provided with a total of 6 sensory pits on each side of the body: 4 forming a row parallel to the lower margin of the pronotum and 2 more central, parallel to the upper margin of the pronotum; mesonotum features 6 sensory pits in total on each side of the body: 2 in the postnotum, parallel to the medial carina, and 4 grouped in pairs in the alinotum, one pair more central and the other in the lateral margin of the segment; metanotum with a total of 2 sensory pits located in the metathoracic alinotum. Metathoracic postnotum with 3 pores





**Figure 4.** *Valenciolenda fadaforesta* I–V nymphal instars. Top side left to right: nymphal instars I (N1), II (N2) and III (N3); bottom side left to right: nymphal instars IV (N4) and V (N5). Habitus in dorsal view.

on each side, parallel to the inferomedial margin of the metanotum. Tergites of the abdominal segments with a higher degree of sclerotization. Segments I–II smaller in size. Segments I–IV with a small row of tubercles on the lower margin, part of the cuticle sculpture. Sensory pits arranged in the abdominal segments as follows: segments IV–VII with a row of 3 sensory pits on each side of the body, located at the most distal ends of the segments. Segment VIII with a single small sensory pit on each side of the body. Segments IX–XI forming the genital complex (Fig. 4).

### **Nymphal instar III (N3)**

Total body length between 1.5–1.7 mm (n=13). Antennae furnished with 6 plate organs, 5 on the pedicel and 1 on the third antennomer. Tergites of the thoracic nota well sclerotized, barely separated from each other. Pronotum with 1 well-marked intermediate carina on each side of the body, separating the anterodorsal region of the pronotum, hardly visible from the dorsal perspective of the body, from the triangular dorsal region. Meso- and metanotum with 2 medial carinae on each side of the body dividing the segments in postnotum and alinotum. Small wing pads present in the mesothoracic alinotum, with a longitudinal carina. Vestigial expansion of the hind wing pads in the metathoracic alinotum. Sensory pits arranged in the thoracic nota as follows: pronotum shows a total of 9 sensory pits on each side of the body, arranged in rows: 4 forming a row parallel to the upper margin of the pronotum and 5 forming another row parallel to the lower margin; mesonotum provided with a total of 11 sensory pits on each side of the body: 4 in the postnotum, forming a row parallel to the medial carina, and 7 in the alinotum (3 in the central region of the alinotum and 4 in the lateral margin following the longitudinal carina); metanotum with a total of 5 sensory pits located in the metathoracic alinotum: 2 parallel to the medial carina and 3 in the lateral region of the alinotum. Metathoracic postnotum with 7 pores on each side, irregularly arranged across notum, bilaterally and individually variable. Completely sclerotized abdominal segments. Segments I–II smaller in size. Segments I–IV with a small row of tubercles on the lower margin, part of the cuticle sculpture. Sensory pits arranged in the abdominal segments as follows: segments IV–VII with a row of 4 sensory organs on each side of the body, located at the most distal ends of the segments. Segment VIII with a single small sensory pit on each side of the body. Segments IX–XI forming the genital complex (Fig. 4).

### **Nymphal instar IV (N4)**

Total body length between 2.4–2.7 mm (n=14). Antennae with 7 sensory plate organs, 6 on the pedicel and 1 on the third antennomer. Tergites of the thoracic nota well sclerotized, barely separated from each other. Pronotum with 1 well-marked intermediate carina on each side of the body, separating the anterodorsal region of the pronotum, hardly visible from the dorsal perspective of the body, from the triangular dorsal region. Meso- and metanotum with 2 medial carinae on each side of the body

dividing the segments in postnotum and alinotum. Larger wing pads present in mesothoracic alinotum, provided with 2 longitudinal carinae. Vestigial expansion of the hind wing pads in the metathoracic alinotum. Sensory pits arranged in the thoracic nota as follows: pronotum features a total of 10 sensory pits on each side of the body, arranged in rows: 5 forming a row parallel to the superior margin of the pronotum and 5 forming another row parallel to the lower margin; mesonotum possesses 20 sensory pits in total, arranged on each side of the body: 6 (5 + 1) in the postnotum, forming a row parallel to the medial carina, and 14 in the alinotum (3 in the central region of the alinotum, 4 forming a row parallel to the first longitudinal carina and 7 (4 + 3) forming two rows between the second longitudinal carina and the lateral margin of the mesonotum); metanotum with 6 sensory pits in total, located in the metathoracic alinotum: 3 grouped parallel to the medial carina and 3 forming a row in the lateral region of the alinotum. Metathoracic postnotum with 10 pores on each side, irregularly arranged across notum, bilaterally and individually variable. Completely sclerotized abdominal segments. Segments I–II smaller in size. Segments I–IV with a small row of tubercles on the lower margin, part of the cuticle sculpture. Sensory pits arranged in the abdominal segments as follows: segments IV–VII provided with a row of 5 sensory pits on each side of the body, located at the most distal ends of the segments. Segment VIII with 3 small sensory pits on each side of the body. Segments IX–XI forming the genital complex (Fig. 4).

### **Nymphal instar V (N5)**

Thoroughly described by Hoch et al. (2021) with a supplementary description provided here. Total body length between 2.8–3.2 mm (n=8). Antennae furnished with 10 sensory plate organs, 9 in the pedicel and 1 in the third antennomer. Fully developed wing pads in the mesothoracic alinotum, with 2 longitudinal carinae. Metathoracic alinotum not visible, hidden under the mesothoracic wing pads. Metanotum with 3 visible sensory organs located in the metathoracic alinotum, parallel to the medial carina. Metathoracic postnotum with 15 pores on each side, irregularly arranged across notum. Completely sclerotized abdominal segments. Segments I–IV with a small row of tubercles on the lower margin, part of the cuticle sculpture (Fig. 4).

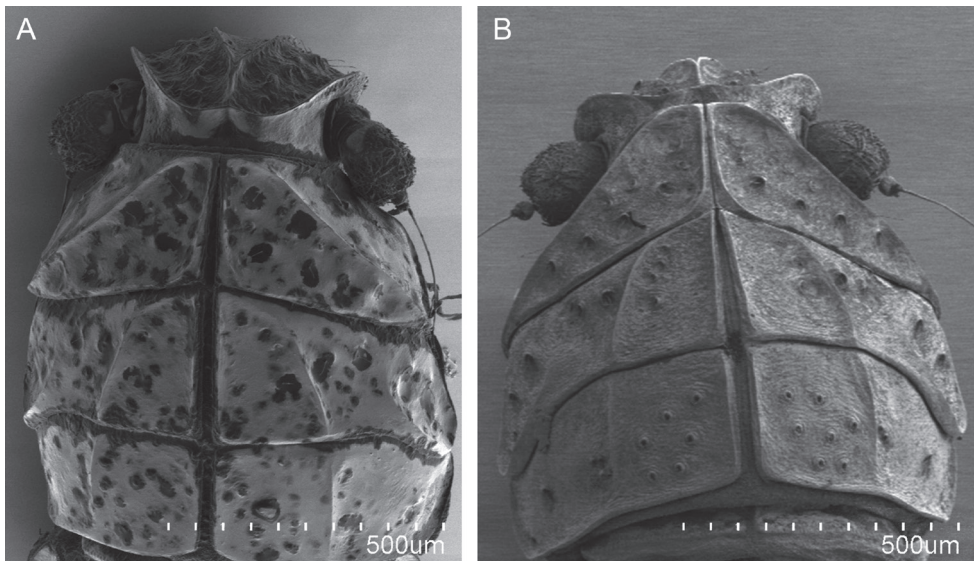
### **Discussion**

The observable changes between the different nymphal stages of *Valenciolenda fadaforesta* consist of an increase in body size, an increase in the number of sensory pits and wax-secreting pores present in both the thorax and the abdomen, and the development of wing pads that develop into wings once they have reached maturity. The increase in length of the metathoracic tibiae and femora, as well as in the number of sensory pits and wax-secreting pores present in the tergites of the thoracic segments, have been the diagnostic characters used for the separation and classification of

the different nymphal stages. The structures studied in the cuticle of *V. fadaforesta* consist of sensory organs composed by wax filaments located in small pits, distributed throughout the thoracic region and in the caudal segments of the abdominal region, as well as small wax-secreting pores located in the postnotum of the metanotum. These individuals secreted a waxy substance that filled the cavities of the cuticle, made it difficult to study the structures and hide the pits where the wax filaments are located. So, it is possible that some of the smaller pits have gone unnoticed. The clear visibility of the wax-secreting pores present in the metathoracic postnotum has been very useful to differentiate between the smaller nymphal stages. It should be noted, however, that there exists some interindividual and intraindividual variability, with specimens that present a variable number of wax-secreting pores on both sides of the thorax (Fig. 5).

The life cycle of this species is unknown. Currently, we have no recorded evidence on the duration of single instars, overall lifespan, copulation, oviposition, or the eggs themselves. Furthermore, the literature on nymphal morphology and its development in the Kinnaridae family is also lacking, and the taxon has not been examined in the studies on nymphal morphology of the fulgoromorphs by Yang and Yeh (1994) and Emeljanov (2001) due to the lack of material.

The life cycle of *V. fadaforesta* is an hemimetabolous cycle composed of five nymphal stages and the adult stage. Juvenile stages of hemimetabolous insects resemble adults, except for the absence of functional wings and immature genital configuration. In these life cycles, embryogenesis gives rise to nymphs that possess adult characteristics.



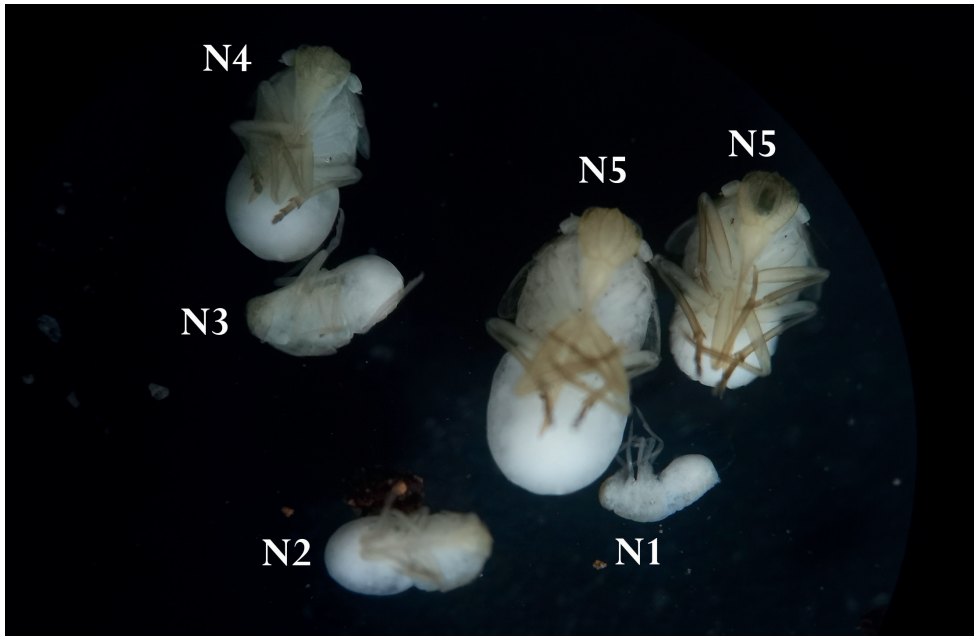
**Figure 5.** Nymphal instar III of *Valenciolenda fadaforesta* (SEM). Thorax in dorsal view **A** specimen 1 **B** specimen 2. Note variation among individuals. In specimen 1 a waxy substance covers the tergites, absent in the specimen 2. The variation in the number of structures present in the same organism can also be observed in specimen 2, with 7 pores in the left section and 8 on the right.

Development of these nymphs consists of growth in size during which the growth ratio of the linear dimensions of the exoskeleton tends to remain constant between nymph-to-nymph molts (Bellés 2020). Nymphs gradually develop into adult characteristics, with the final moult bringing forth the adult which has functional wings and fully developed external genitalia.

In *V. fadaforesta* the growth and development of the nymphal stages seems to follow a linear progression according to what can be observed (see Fig. 2). The increase of overall body length and proportions of femora/tibia length is more or less gradual from instar I – IV, while the change from instar IV –V is notably more distinct. Although we attempted to collect individuals across all possible sizes, these were small insects with tiny variations in size between different nymphal stages, which were difficult to discern with the naked eye. Apparently, overall body length alone is not sufficient to discriminate the nymphal stages, as it notably varies among individuals of the same instar. The observed variation may be due to genetic variation or may be due to individual modification during ontogeny (freshly hatched nymphs of a given instar are likely to be smaller than nymphs closer to the consecutive molt), or even caused by physiological condition (e.g., starved vs. well-fed). The cuticle in nymphs is not fully hardened, even in sclerites, and especially intersegmental membranes are rather extensible. The correct nymphal stage can ultimately only be determined upon examination of cuticle structures and measurement of the legs. The base pattern of the fulgoromorphan Hemiptera consists of five nymphal stages, with the only exception of *Lycorma delicatula* (White, 1845) (Fulgoridae) which has only four (Dara et al. 2015). Our observations on the nymphal development of *V. fadaforesta* are consistent with the existing literature on this Hemiptera taxon (Liang 2001; Hoch and Ferreira 2016; Hoch et al. 2021) and other closely related taxa (Wilson and Tsai 1982; Chen and Yang 1995; McPherson and Wilson 1995).

In the samples examined, individuals belonging to the same nymphal stage can be observed showing great differences in the body size and size of the abdominal region. When observed under a stereomicroscope, some individuals had a more “swollen” abdomen, although the size of the thorax and the cuticle that covers the abdominal tergites maintained its proportions (Fig. 6). At first glance, this variation in the size of the abdomen could result in a misclassification of the nymphal stages. Thus, measurement of its legs and the confirmation of key structures in its cuticles (i.e., presence or absence of wing pads, relative size of wing pads, number of sensory plate organs on the pedicel, number and distribution of sensory pit organs on pronotum, mesonotum and metanotum and number and distribution of wax-secreting pores) are required.

Regarding the size of nymphs, there was some variation of overall body size among individuals of the same nymphal instar, perhaps due to physiological conditions and individual development. Phenotypic variation in growth rates was present in all living organisms, and their plasticity was affected by multiple environmental variables where temperature and food availability were most influential (Lee and Roh 2010). Regarding temperature, underground habitats are one of the few natural systems where microcli-



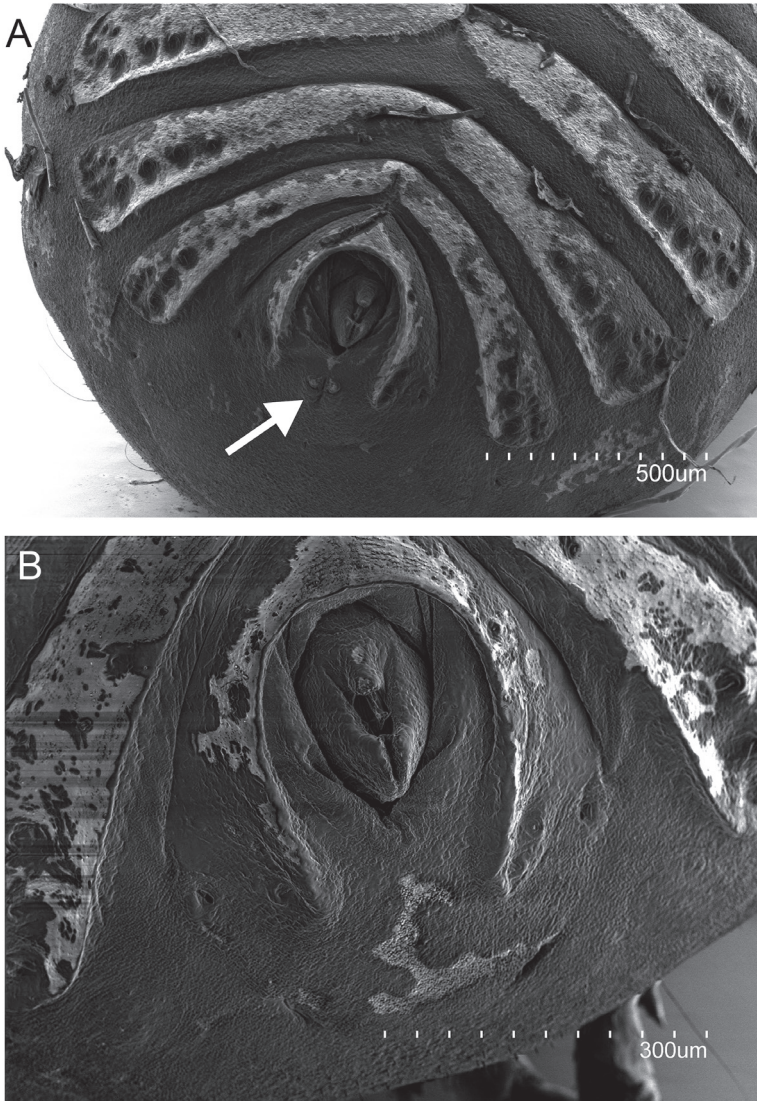
**Figure 6.** Body size variation in nymphal instars I–V (stereomicroscope photograph). Nymphal instar V are largest but display high degree of variation in overall abdomen size (see text for possible explanation).

matic conditions can reach levels of homogeneity otherwise only achieved under laboratory conditions. The stable conditions in the deep cave zone thus rule out temperature oscillations as the determining factor underlying the observed phenotypic variation among individual nymphs of the same instar, e.g., in total body size (Sánchez-Fernández et al. 2018). The section of the cave where most of the nymphs were collected, the so-called “Root Room” (or *Sala de las Raíces*) in Murciélagos Cave, is no exception, with little variable temperatures between 16.5 °C and 18.3 °C throughout the year (Sendra et al. 2015). The variations in the size of the collected individuals could then be explained by differences in the stage of development or by differential access to the food source.

The gradual increase of absolute and especially relative length of the hind-tibia in consecutive instars may be indicative of increasing mobility in the course of nymphal development. Field observations of another obligately cavernicolous planthopper species, although in a different taxon of family rank, *Oliarus polyphemus* (Cixiidae) from lava tubes on Hawaii Island, revealed that earlier instars (I–III/IV) appear to be rather sedentary, sitting on roots and feeding. Older instars however, especially instar V, show dynamic mobility patterns, actively moving away from roots, most likely in search of a secure place suitable for molting into adults (Hoch, personal observation; also see Hoch and Howarth 1993). It is conceivable that nymphs of *Valenciolenda fadaforestae* show similar mobility patterns.

To discern the sexes of immatures is challenging. Nymphal instars I–IV do not present sexual structures that allow clear differentiation between male and female specimens. In the fifth nymphal stage, two different genital phenotypes exist: in some, yet not all, V instar nymphs we found two inconspicuous conical processes in the

posteroventral region of segment IX (Fig. 7). These were only detectable under high magnification (SEM), and could neither be seen with the naked eye or even with the use of a stereomicroscope. These conical processes have been interpreted by Hoch et al. (2021) to be either precursors of the genital styles of the male or the gonocoxae VIII of the female. Sexing immatures of *Valenciolenda fadaforesta* is further impeded by the fact that highly modified external female genitalia are characteristic for the Kinnaridae (as well as the closely related taxon Meenoplidae), and even females of epigean species display strongly reduced ovipositors (Wilson 1983).



**Figure 7.** Nymphal instar V of *Valenciolenda fadaforesta* (SEM). Tip of abdomen in dorsodistal view. Conical processes (**A**, arrow) located medially at the level of the posteroventral corner of tergite IX present in **A** and absent in **B**.

## Conclusions

The results and observations presented here are based exclusively on field samples. These first ever descriptions of all nymphal instars of a species of the Kinnaridae add to the existing knowledge of postembryonic development in this taxon, and further contribute to the reconstruction of ground pattern, or body plan, of Fulgoromorpha nymphs. It should be kept in mind, however, that the study organism, *Valenciolenda fadaforesta*, is an obligate troglobiont, and thus a highly specialized species. Certain morphological configurations may have evolved as reductive characters and may thus represent autapomorphies for this particular species rather than for the entire family. Since *Valenciolenda fadaforesta* is the only representative of the Kinnaridae of which nymphs have been available for study, a comparison with nymphs of epigean Kinnaridae species has not been possible. Accordingly, we cannot with certainty distinguish between potential autapomorphies for the species and those for the entire lineage. Suspected autapomorphies for *V. fadaforesta* are the absence of compound eyes in all instars, and perhaps related to it, the extremely short and wide vertex. Similar alterations of head capsule architecture have been observed in other cavernicolous Fulgoromorpha. Hoch and Howarth (1989), in a study on cavernicolous Cixiidae from Australia, hypothesized that “the broadening of the vertex [...] is apparently correlated with the reduction of compound eyes, and might have resulted from mechanical competition within the head capsule during morphogenesis, analogous to that which Berger and Howard (1968) reported for birds”.

Given its occurrence in a specialized habitat yet in a comparatively easily accessible location, in the vicinity of academic facilities, *Valenciolenda fadaforesta* is a potential model organism to study morphogenesis in an obligately cavernicolous insect. In order to develop a protocol for rearing *Valenciolenda fadaforesta* in the laboratory, further studies are required to understand life history, longevity, reproduction rate, and host associations as well as to determine environmental factors controlling population size. Information on these will also be pivotal in developing strategies for management and conservation of the caves and other organisms inhabiting them.

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