



Pathogenic DWV infection symptoms in a Cretaceous cockroach

by

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with 3 text-figures

Abstract

Unequivocal palaeontological evidence for viruses is usually absent. A specimen of the extinct predatory cockroach *Stavba babkaeva* gen. et sp. n. from Cretaceous Myanmar amber (98 Ma) shows symptoms of Deformed Wing Virus infection caused by pathogenic DWV-*Iflavirus*. The hindwing is undeveloped and both curled forewings are symmetrically deformed, differing from environmentally caused asymmetries known from Pripyat and Fukushima. While some unknown cockroach mutation might have the same symptoms, ontogenetic defects (such as incomplete moulting) differ in complete lack of sclerotization, modified forewing bases and presence on both wings. Post-depositional, taphonomic influence can be excluded due to local character of the deformation (forewings on both sides) while other areas are undeformed. Drying shrinking can be excluded due to brittle character of the wing, which would crack instead – and it could not, be local either. Pathogenic RNA-viruses probably circulated among vertebrates and invertebrate decomposers/predators in the dinosaur-age ecosystems. Our discovery complements an indirect putative evidence of Retrovirus infection that modified dinosaur bones.

Keywords: fossil insect, Mesozoic cockroach, Myanmar, Cenomanian amber, ancient virus

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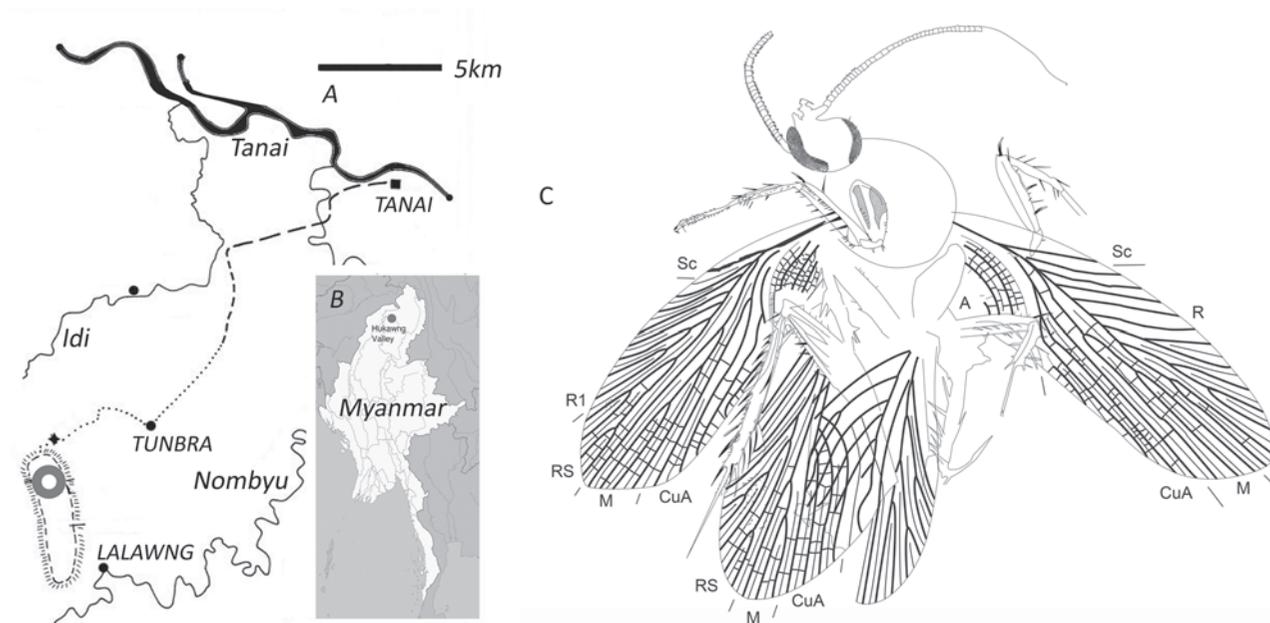
Introduction

Past ecosystems are becoming comparatively well investigated taxonomically (~110,000 inclusions in Burmite only), but structural information regarding function and interconnection of species is lacking. Cockroaches are abundant inclusions in amber. Their diversity in Burmese amber is comparable to that of modern rainforests based on 527 specimens in 74 species. Disparity of forms is even higher with poisonous species (ŠMÍDOVÁ & LEI 2017), crane-fly-like predators (VRŠANSKÝ & BECHLY 2015), and extinct groups present (BAI et al. 2016, 2018, VRŠANSKÝ & WANG 2017, KOČÁREK 2018, PODSTRELENÁ & SENDI 2018)). The family Liberiblattinae is represented by 14 genera and 15 species in 25 specimens. A new predatory taxon *Stavba babkaeva* VRŠANSKÁ et VRŠANSKÝ, gen. et. sp. n. described herein differs from all other dictyopterans (cockroaches and their relatives) in having primitively raptorial legs with asymmetrically distributed spines on the front femur (see Text-fig. 1c and LEE (2016), DITTMANN et al. (2015) for analogical structures in unrelated predatory cockroaches) and pale colour with reduced pigmentation, one specimen shows infection symptoms. The

aim of the present paper was to formally describe the cockroach possessing the insect-specific (+)ssRNA *Iflavirus* symptoms – the earliest and first significant specific record of any virus infection.

Geological settings

The Burmite amber pieces were obtained from an amber mine in the Hukawng Valley, Kachin Province, northern Myanmar (see Text-fig. 1 and KANIA et al. 2015). The matrix has been dated as mid-Cretaceous/earliest Cenomanian with an absolute maximum age of 98.8 ± 0.6 Ma established by U-Pb dating of zircons (SHI et al. 2012, GRIMALDI et al. 2002, ROSS et al. 2010). Cockroaches constitute 2–5 % of the insects from this deposit (GRIMALDI et al. 2002, ROSS et al. 2010, ZHERIKHIN & ROSS 2000) with some 4,000 specimens collected (VRŠANSKÝ & WANG 2017). Three samples are deposited in Nanjing Institute of Geology and Paleontology, Chinese academy of Sciences. Additional supporting specimens are deposited in Dian Jiang Collection in Heshun Town, Yunnan Province, China (Lv JUN, curator, LV177) and Zhejiang Museum of Natural History, Hangzhou, China (HAO WU, curator, ZMNH M6898).



Text-fig. 1. Location map within (North) Myanmar (A, B) Hukawng Valley, Kachin state (Copyright of map: UWE DEDERING, Wikimedia Commons, under free GNU/CC licence, and modified after CRUICKSHANK & KO 2003) and dinosaur-aged (98Ma) predatory cockroach *Stavba babkaeva* sp. n. (Liberiblattinae) in Myanmar amber. NIGP164831 normally developed adult ?male holotype.

Material and Methods

Photographs were taken using a Zeiss Stereo Discovery V16 microscope system and Zen software with incident and/or transmitted light. Focus stacking was done with Combine ZP and 20–50 images of different focal plane. Figure plates were prepared using CorelDraw X4 and Adobe Photoshop CS3. The terminology of the wing venation follows COMSTOCK & NEEDHAM 1889). Fore description details see (DELCLÒS et al. 2016, VRŠANSKÝ 2004, MARTIN 2010, BARNA 2014, VRŠANSKÝ et al. 2017, GRIMALDI 2003, HÖRNIG et al. 2013, 2017, ORUŽINSKÝ & VRŠANSKÝ 2017, GAO et al. 2018, LIANG et al. 2018).

Results

Fossil cockroaches (n = 110,000, 4,000 from Myanmar amber) with developmental deformations affecting the wing structure are unknown. The paratype specimen (NIGP164832) bears symmetrical deformation of both fully developed but curled and shrunken forewings. Main veins, intercalary veins as well as crossveins are distinct. Distinct is also a local melanization of the membrane (for all these characters see Text-fig. 2b2). Other parts of the body are undeformed. Hindwings are entirely missing. These deformations correspond to deformation of insect wings caused with Deformed Wing Virus *Iflavirus* (see Discussion).

Systematic paleontology

Class Insecta L., 1758

Order Blattaria LATREILLE, 1810

Family **Liberiblattinae VRŠANSKÝ, 2002**

Genus *Stavba* VRŠANSKÁ et VRŠANSKÝ, gen. n.

Type species: *Stavba babkaeva* VRŠANSKÁ et VRŠANSKÝ, sp. n. described below by monotypy.

Derivation of name: after *stavba* (Slavic for building).

Text-fig. 2. Dinosaur-aged (98Ma) predatory cockroach *Stavba babkaeva* sp. n. (Liberiblattinidae) in Myanmar amber (a–b, d, f) and living *Blattella germanica* (c). a NIGP164831 normally developed adult *Stavba babkaeva* male, b NIGP164832 adult *Stavba babkaeva* female showing (+)ssRNA *Iflavirus*-infection symptoms with detail on the right forewing below fly and fern-like inclusion (b2), c fully melanised and sclerotized living *B. germanica* with incomplete moulting deformation, d phoretic pseudoscorpion on antenna of an unidentified cockroach inclusion, e unsclerotised and freshly moulted recent cockroach. Scales 1 mm.

Differential diagnosis: Differs from other genera of this family in very small size, pale colouration (except for legs with dark longitudinal stripes), foreleg asymmetrically raptorial with much limited cursorial function, and short forewing Sc.



Remarks: Wide veins with wide intercalaries, characteristically curved forewing veins and the general corydioid hindwing venation allow placing of the new taxon in the clade Corydioidea + Mantodea. The head with predatory modifications, in being orthognathous with huge eyes and small ommatidia, resembles that of the mantodean family Juramantidae (c.f., DELCLÒS et al. 2016: figs 7–1, 8–1). The forelegs being functionally nearly completely raptorial indicate affinities with mantodeans, but the short forewing Sc and asymmetrically located spines on forelegs suggest indirect relationship and a possible convergence (c.f., Eadiidae VRŠANSKÝ, 2009, Manipulatoridae VRŠANSKÝ & BECHLY, 2015, Mutoviidae VRŠANSKÝ & ARISTOV, 2010, *Raptoblatta* DITTMAN et al., 2015 in Mesoblattinidae HANDLIRSCH, 1906–1908 (LEE 2016)). The modified Sc allows placement within Liberiblattinidae VRŠANSKÝ, 2002. The transparent membrane is unusual, but is found also in primitive (J1, J/K) *Elisamoides* (VRŠANSKÝ 2004, MARTIN 2010) from Australia and Mongolia, which differs in possessing branched A and distinctly branched Sc. A similar undescribed representative of the family is also reported from Chernovskie Kopi (J/K) in Russia (BARNA 2014: fig. 4G). Another transparent membrane taxon is *Eublattula* HANDLIRSCH, 1939 (J1) from Germany and England, possessing a strongly branched A.

The preserved immature belongs to the last pre-molting stage as evidenced by fully developed wing pads. Preservation of another specimen of the same stage (LV177) suggest the species might already be rare, but autochthonous.

Description: as for species.

Stavba babkaeva VRŠANSKÁ et VRŠANSKÝ, sp. n.

Holotype: NIGP164831. Nearly complete adult ?male.

Paratype: NIGP164832. Complete adult female with syndroms of (+)ssRNA *Iflavirus* infection.

Additional material: NIGPP003 (male) and LV177 (female). Complete preimaginal immature stages. M6898. Complete early immature.

Derivation of name: babkaeva is from babka (Slavic for grandmother) and Eva (grandmother of L.V.)

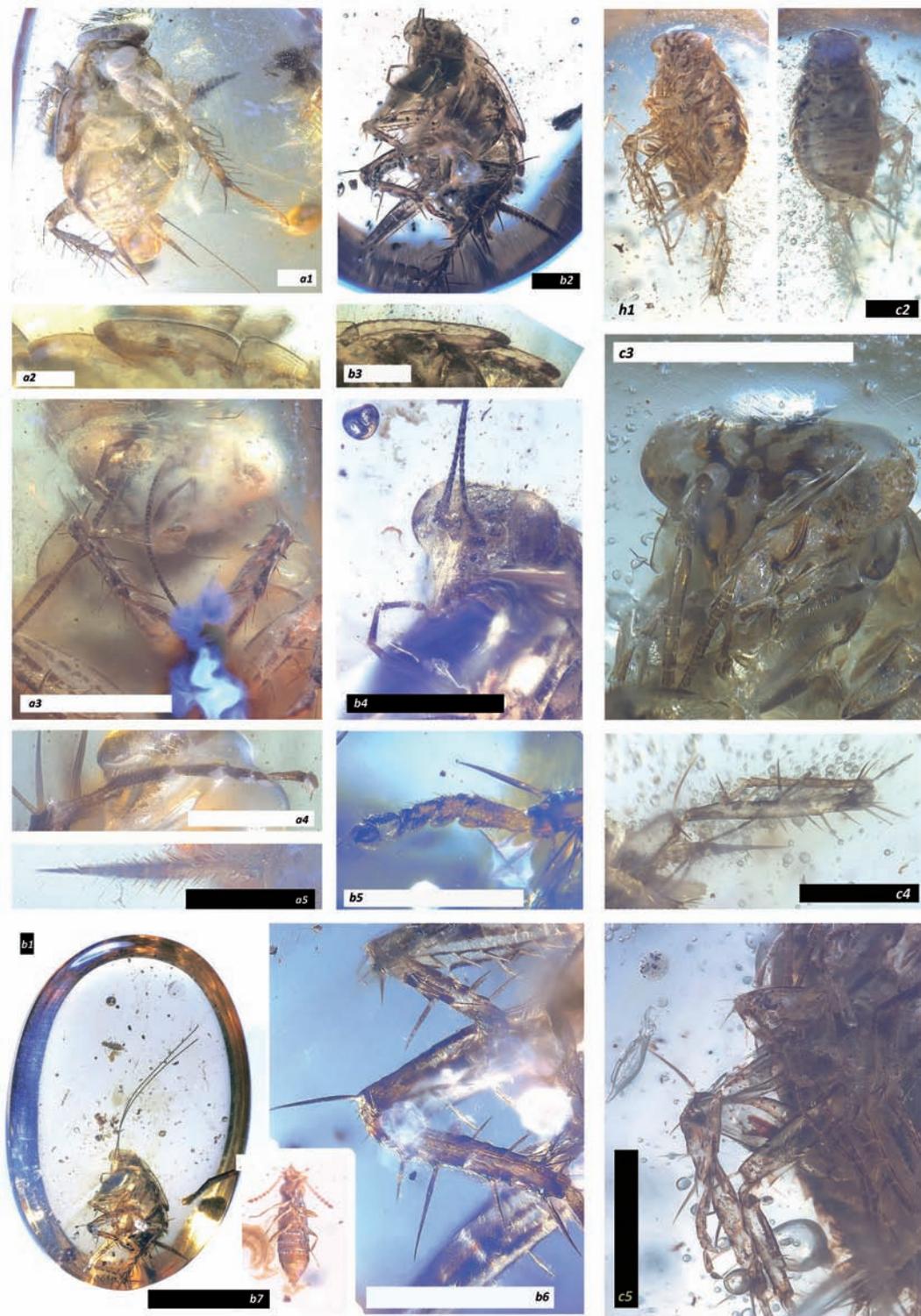
Character of preservation: 2 complete adults (1 male, 1 female) and (preimaginal) immature male and 2 females.

Type locality: Hukawng valley, Myanmar.

Type Horizon: Cenomanian Late Cretaceous.

Description (based on holotype): Although head partially decomposed, clearly not fully hypognathous with huge, oval, elongated narrow eyes (covering more than 4/5 of the head length) with numerous very

small ommatidia (at least 600). Long sensillae sparsely distributed over eye surface. Antennae not preserved completely (26 completely preserved right segments/38 left segments) but apparently rather short (ca. 5 mm long) with 60 short, wide segments partially preserved (probably not more than 70 in total due to rapid narrowing towards of last preserved segments). Each antennal segment with short, wide chaetia distributed in up to 4 rows. Pronotum nearly circular, only slightly transversal (2.1 × 1.8 mm) and slightly vaulted, nearly planar. No sensillae covering surface or on margin. Forelegs raptorial, strongly asymmetrical. Right coxa very long and wide (0.8 × 0.3 mm) with only sporadic short, fine sensilla chaetia and two slightly dark stripes. Trochanter curved, very long (0.2 × 0.1 mm) with 3 long sensillae. Femur comparatively narrow (1.3 × 0.2 mm), terminating with 2 apical spurs, posterior margin with incision covered by two unidentical and asymmetrical rows of strong spurs (distal 3 very long strong spurs basal series of 14 strong but short spurs, distally 7 small spines and proximally 4 very strong, medium-size spurs). Tibia medium-size (1 × 0.1 mm), with only about 9 spurs, but with numerous sensillae adjacent to femur. Tarsus very long (ca. 1 mm) with pulvilli on each segment and small arolium near symmetrical short claws. Left foreleg notably robust with major spurs distributed unevenly and irregularly with small spurs among them and tibia with fewer spurs. Mid legs cursorial, but femur (ca. 1.2 mm long) not very strong and with posterior ridge with two rows of spines (at least 5 + 5), tibia strong with at least 10 spurs. Hind leg basically cursorial, long, strong, with numerous spurs, femur very strong and wide (2 × 0.6 mm) with posterior ridge with 2 rows of strong spurs alternating with smaller spurs and sensillae, tibia very long 2.5 × 0.3 mm, with at least 17 spurs. Forewing elongated (right: 5.1 × 1.8 mm, left: 5.3 × 1.9 mm; area 6.83 mm²), transparent, with unparallel margins in the basal third and with sharp distal apex. Intercalaries present all over the wing, cross veins restricted to clavus (where dense) and sporadically present in apical half. Sc in right forewing very strong, simple, very short, in left forewing simple and indistinct, similar to first radial branch, R1 with 7–10 veins at margin with few primary branches (only secondarily branched vein is the posteriormost R1 stem), “RS” differentiated (2–4 veins at margin), M with 3 or 4 straight medium sized branches, CuA branched basally with 4 extremely long branches. CuP (clavus) short, probably with only 4 curved simple A veins.



Text-fig. 3. Dinosaur-aged (98Ma) fully sclerotized immature stages of a predatory cockroach *Stavba babkaeva* sp.n. (Liberi-*blattinidae*) in Myanmar amber a1-5 NIGPP003 (dorsal view on specimen, wing pads, ventral side, fore tarsus and left cercus of a) preimaginal immature male, b1-7 LV177 (dorsal view on specimen, wing pads, ventral side, mid right tarsus, all legs with “raptorial” spines and a syninclusion beetle of a) preimaginal immature female, c1–5 M6898 early stage. Scales 1 mm.

Hindwing transparent, approximately (articulation indistinct) of identical length to forewing with intercalaries and numerous cross veins throughout the membrane, apex rounded. Left hindwing is damaged (possibly due to predation), cracked and folded (partly removed apparently prior to deposition), but no deformities (see 50) occur on the membrane. Sc simple and straight but very short, R1 apparently differentiated with 1 basally or terminally branched vein, RS occasionally dichotomised, R with 7 or 8 veins at margin, M simple (left) or with 3 straight, long branches, CuA with one secondarily branched vein (second one posteriormost) and 8 or 9 veins at margin, CuP apparently simple.

Description of immature specimens (NIGPP003 specimen measurements in parentheses, Text-fig. 3a [LV177 specimen measurements in square parentheses], Text-fig. 3b) reveal important details inaccessible on adults. They can be categorized within the present species on the basis of generally white color, thin colored lines on legs, antennal sensillae and general morphology of partially raptorial forelegs with asymmetrically distributed movable or immovable (asymmetrically distributed) spines, and mid and hind femora with certain “raptorial” modifications similar to those in *Santanmantis axelrodi* GRIMALDI, 2003 (GRIMALDI 2003, HÖRNIG et al. 2013, 2017) likely dysfunctional, resulting from homeotic genes actions). Pre-imago stage evidenced with partially colored wing buds. Species developed melanin control in immature stages and during last molt. Head [0.62×1.8 mm, 1.66 mm high] near white, unmelanized (melanized in adults), characteristic dark head stripes distinct but weak. Antenna [0.59 mm wide with 0.4 mm long sensillae] identical with adults, eyes [0.72×0.43 mm] incompletely melanised. Facets comparatively small (nocturnal lifestyle excluded), lateral and central ocellus lentiform, large, transparent, distinct. Sclerotised parts and appendages melanized in part. Palps 4-segmented (segments ?/ 0.25/ 0.25/ 0.2 mm long) [0.12/ 0.25/ 0.35/ 0.32 mm], labial ones 2- or 3-segmented (segments ?/ 0.2/ 0.2 mm long) [?/ 0.14/ 0.24]. Pronotum strongly transversal (1×2.8 mm) [0.92 \times 2.4], widest in the very basal part, narrow (2 mm) in the distal part, white with pale brown colorations. Wing buds longitudinal (2×0.8 , 2×0.8 mm) [2.7×0.7 , 2.5×0.6 on left side, 2.4×0.7 , 2.4×0.6 on right side] with distinct wide and dark costa and dark apex. Legs medium-sized, all 3 femora pairs strong and wide (0.8×0.2 , 1.8×0.7 , 2×0.5 mm) [1.51×0.24 , 1.76×0.32 , 2.26×0.51 mm on right side],

with terminal spine 0.56 mm long [0.56], heavily carinated with spurs having helical ridges (appearing somewhat raptorial – foreleg apparently raptorial with 8 asymmetrically distributed spines), tibiae (0.8×0.2 mm, 1.6×0.2 , 2×0.2 mm) [0.84×0.19 , 1.37×0.27 , 2.97×0.2 mm on right side] strongly carinated, hind tibia with 20 spurs. Tarsi with dense sensilla, long, tarsomeres 1–4 (1/ 0.4/ 0.3/ 0.2/ 0.7 mm long) [0.46/ 0.14/ 0.17/ 0.14/ 0.33] with distinct pulvilli and the symmetrical terminal claw (0.2 mm long) [0.2] possesses a fully developed big arolium.

Body 2.9 mm wide, thick, with 10 segments preserved, pale, with pale brown transversal stripe on each segment. Cercus very narrow (2.1×0.4 mm) [1.9/ 0.15], 11-segmented, with 0.2 mm [0.27] long (short) sensilla. Long sensilla of most *Liberiblattinidae* and *Umenocoleidae* are also present.

Discussion

Reason of forewing deformation might be 1) taphonomical, 2) incompleting moulting process, 3) genetical indisposition, 4) developmental deformation, 5) virus infection. (1) While deformed are only forewings on both sides of the body, and other parts of body are unaffected, the **taphonomical** processing resulting from the “flowing” or melting of amber can be without any doubts excluded. Additionally, hindwings are entirely undeveloped, which excludes local but selective shrinking resulting from physical properties of the wing membrane. Additionally, the wing is brittle, so it will rather rupture than shrink. Combination of local character of deformation, different deformation of fore and hindwings (and absence of similar even partial deformation in more than 4,000 samples) sufficiently excludes the taphonomic effects. (2) Completely undeveloped hindwings exclude the possibility of **unfinished moulting**. Because moulting in cockroaches is characteristic with melanisation following only after the both wings are outstretched, moulting can be excluded additionally also with the apparent sclerotization and partial melanisation (Text-fig. 2b2). (3) While **genetical indisposition**, namely the mutation influencing the cascade of virus-binding receptors would cause the same phenotypic effect, probability of such change is extremely low (as it has to influence different genes simultaneously) and this is unknown in living insects, this possibility is disregarded in this study. In *Drosophila melanogaster* vestigial mutations have yielded varying phenotypes from complete loss of wings (and haltere) to narrow, nicked,

or scalloped wings (BOWNES 1981). These mutations are not accompanied, however, by change of overall body pigmentation, which require other mutations such as hormonal. Bursicon is a peptidic hormone (KOSTRON et al. 1996), controlling wing expansion, tanning and hardening of cuticles, events finalizing adult appearance in *D. melanogaster*. Bursicon hypomorphic mutants fail to inflate wings after eclosion (FRAENKEL & HSIAO 1962, COTTRELL 1962, DEWEY et al. 2004). Furthermore, flies lacking both CCAP and bursicon peptide show much more severe defects at ecdysis than do mutants lacking one neuropeptide as bursicon may be able to rescue some CCAP functions, but not vice-versa (HARWOOD et al. 2014). Also, bursicon activity is mediated through its cognate G protein-coupled receptor (GPCR), known as rickets (LUO et al. 2005). Mutants for rickets are viable and molt but have abnormal post-ecdysial behaviour. Rickets in insects results in twirled femurs, crossed post-scutellar bristles, shrunken wings as well as defects in tanning and melanization. This mutation is also observed in flies that have had their eclosion hormone (EH) cells genetically killed, implicating EH in the regulation of post-ecdysial expansional behavior as well as of ecdysis itself (BAKER & TRUMAN 2002). These symptoms are currently unknown in cockroaches. (4) **Environmentally induced deformities** in recent insects (Chernobyl in Russia and Fukushima in Japan) are asymmetrical (HESSE-HONEGGER 2001, HIYAMA et al. 2012, 2013, TAIRA et al. 2016). Moulting in cockroaches is incomplete and an asymmetrically deformed wing due to an interrupted last moult can be frequently observed such as reported recently in antlions (EISELE 2008). A symmetrically deformed Two-lined Leatherwing Beetle (*Atalantycha bilineata*) has also been reported (SHELLY 2016).

We thus propose a direct **virus infection symptoms** and because no other known viruses deform insect wings, we suggest Deformed Wing Virus (DWV) *Iflavirus* (additionally causing symmetrical deformities observed herein). *Iflavirus* is known in fifteen arthropod species (ICTV Virus Taxonomy 2016 Release, retrieved 30 August 2017, SILVA et al. 2015). Infectious flacherie virus (the type species) affects the silkworm *Bombyx mori* (AIZAWA et al. 1994). The best-studied DWV infection (10140 nucleotides and the poly(A) tail (LANZI et al. 2006)) is in honeybees and other bee species like *Bombus terrestris* (GENERSCH et al. 2006) and impairs cognitive functions (IQBAL & MUELLER 2007). It is an important pathogen in honey bees and

one of the discussed causes for colony collapse disorder (CCD) (WILLIAMS et al. 2010). Slow bee paralysis virus also attack bees (BAILEY & WOODS 1974). The sackbrood virus also affects bees and two iflaviruses attacks the bugs *Nilaparvata lugens* (MURAKAMI et al. 2014) and *Lygus lineolaris* (PERERA et al. 2012), the latter with incomplete metamorphosis (hemimetabolans), like cockroaches. Another six iflaviruses attack the lepidopterans *Ectropis obliqua*, *Perina nuda*, *Lymantria dispar*, *Antheraea pernyi*, *Opsiphanes invirae* (SILVA et al. 2015, GENG et al. 2014, CARRILLO-TRIPP et al. 2014), *Spodoptera exigua* (two Iflaviruses, CHOI et al. 2012, MILLAN-LEIVA et al. 2012) and one a parasitic hymenopteran *Dinocampus coccinellae* (DHEILLY et al. 2015). The last occurs in *Varroa destructor*. This type of viruses belongs to the primitive RNA type thus its occurrence in Cretaceous amber is not unexpected.

The minute size of viruses generally **disqualifies them from fossilization** (KATZOURAKIS 2013). Rare paleontological indirect evidence for the existence of pathogenic viruses in the geological past includes putatively modified bones of sauropod dinosaurs from the Late Triassic of Patagonia (CERDA et al. 2014), thought to be caused by a retrovirus (ssRNA-RT type virus) analogous to that causing osteopetrosis in living birds. POINAR & POINAR (2005) even documented putative direct evidence for cytoplasmic (CPV) and nuclear (NPV) polyhedrosis viruses from a biting midge and a sandfly in mid-Cretaceous Myanmar amber.

Most viruses are characterized by high substitution rates, which impede the reconstruction of their evolutionary history (DUFFY et al. 2008). Phylogenetic analyses and palaeontological calibrations of molecular clock estimates for “genomic fossils” suggest a CT-boundary age of 93–45 mya for a retrovirus (polydnavirus) – braconid wasp symbiosis (WHITFIELD 2002) and even a Pennsylvanian age (ca. 310 mya) for insect DNA viruses, which seem to have diversified in the Mesozoic together with their host insects (THÉZÉ et al. 2011).

Phoretic species such as pseudoscorpiones are common in amber, e.g., attached to a cockroach antenna (Fig. 2D), which would allow viral infections. With a single unpublished exception (an adult of Alienopteridae), no mites (but see SIDORCHUK & KHAUSTOV 2018) have been found attached to a cockroach in 3,000 examined amber specimens, thus phoretic pseudoscorpions could be important transmission agents.

Conclusions

As evidenced by common occurrence of immatures and two adults, predatory cockroaches (*Stavba babkaeva* sp. n.) of the family Liberiblattinidae were autochthonous inhabitants of Cretaceous Myanmar amber (98 Ma) forests. A paratype specimen shows symptoms of Deformed Wing Virus infection caused by pathogenic DWV-Iflavirus. RNA-viruses probably circulated among vertebrates and invertebrate decomposers/predators in the dinosaur-age ecosystems.

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Author Contributions

We collected and selected samples (X.R., X.L., P.V., L.Š., T.B., L.J., W.H.), provided the systematic description (L.V., P.V.), compared genetic (M.B., G.B.) and ontogenetic (M.Y.) deformation, reviewed fossil viruses and palaeovirology (G.B.), designed research (P.V.) and wrote the paper (P.V., G.B., M.B., E.J.). All authors commented on the manuscript.

References

- AIZAWA, K., FURUTA, Y., KUTATA, K. & SATO, F. (1964): On the etiologic agent of the infectious flacherie of the silkworm, *Bombyx mori* (Linnaeus). – *Bulletin of the Sericultural Experiment Station* **19**: 223–240.
- BAI, M. et al. (2016): †Alienoptera – a new insect order in the roach-mantodean twilight zone. – *Gondwana Research* **39**: 317–326.
- BAI, M., BEUTEL, R.G., ZHANG, W., WANG, S., HÖRNIG, M., GRÖHN, C., YAN, E., YANG, X. & WIPFLER, B. (2018): A new Cretaceous insect with a unique cephalothoracic scissor device. – *Current Biology* **28**: 438–443.
- BAILEY, L. & WOODS, R.D. (1974): Three previously undescribed viruses from the honey bee. – *Journal of General Virology* **25**: 175–186.
- BAKER, J.D. & TRUMAN, J.W. (2002): Mutations in the *Drosophila* glycoprotein hormone receptor, rickets, eliminate neuro peptide-induced tanning and selectively block a stereotyped behavioral program. – *Journal of Experimental Biology* **205**: 2555–2565.
- BARNA, P. (2014): Low diversity cockroach assemblage from Chernovskie Kopi in Russia confirms wing deformities in insects at the J/K boundary. – *Biologia* **69**: 651–675.
- BOWNES, M. & ROBERTS, S. (1981): Analysis of vestigial^W (vg^W) a mutation causing homoeosis of haltere to wing and posterior wing duplications in *Drosophila melanogaster*. – *Journal of embryology and experimental morphology* **65**: 49–76.
- CARRILLO-TRIPP, J. et al. (2014): *Lymantria dispar* iflavirus 1 (LdIV1), a new model to study iflaviral persistence in lepidopterans. – *Journal of General Virology* **95**: 2285–2296.
- CERDA, I.A., CHINSAMY, A. & POL, D. (2014): Unusual endosteally formed bone tissue in a patagonian basal sauropodomorph dinosaur. – *The Anatomical Record* **297**: 1385–1391.
- CHOI, J.Y. et al. (2012): Complete genome sequence of a novel picorna-like virus isolated from *Spodoptera exigua*. – *Journal of Asia-Pacific Entomology* **15**: 259–263.
- COMSTOCK, J.H. & NEEDHAM, J.G. (1899): The wings of insects. – *The American Naturalist* **33**: 117–126, 573–582, 845–860.
- COTTRELL, C.B. (1962): The imaginal ecdysis of blowflies. Detection of the blood-borne darkening factor and determination of some of its properties. – *Journal of Experimental Biology* **39**: 413–430.
- CRUICKSHANK, R.D. & KO, K. (2003): Geology of an amber locality in the Hukawng Valley, Northern Myanmar. – *Journal of Asian Earth Sciences* **21**: 441–445.
- DELCLÒS, X. et al. (2016): New mantises (Insecta: Mantodea) in Cretaceous ambers from Lebanon, Spain, and Myanmar. – *Cretaceous Research* **60**: 91–108.
- DEWEY, E.M. et al. (2004): Identification of the gene encoding bursicon, an insect neuropeptide responsible for cuticle sclerotization and wing spreading. – *Current Biology* **14**: 1208–1213.
- DITTMANN IL, HÖRNIG M.L., HAUG J.T. & HAUG C. (2015): *Raptoblatta waddingtonae* n. gen. et n. sp. — a roach-like insect from the Lower Cretaceous with a mantodean-type raptorial foreleg. – *Palaeodiversity* **8**: 103–111.
- DHEILLY, N.M. et al. (2015): Who is the puppet master? Replication of a parasitic wasp-associated virus correlates with host behaviour manipulation. – *Proceedings of the Royal Society of London, Series B, Biological sciences* **282**.
- DUFFY, S., SHACKELTON, L.A. & HOLMES, E.C. (2008): Rates of evolutionary change in viruses: patterns and determinants. – *Nature Reviews Genetics* **9**: 267–276.
- EISELE, T. (2008): Ant lion adult. The Backyard Arthropod Project blog < <http://somethingscrawlinginmyhair.com/2008/07/19/ant-lion-adult/> > .
- FRAENKEL, G. & HSIAO, C. (1962): Hormonal and nervous control of tanning in the fly. – *Science* **138**: 27–29.
- GAO, T.P., SHIH, C.K., LABANDEIRA, C.C., LIU, X., WANG, Z.Q., CHE, Y.L., YIN, C.C. & REN, D. (2018): Maternal care by Early Cretaceous cockroaches. – *Journal of Systematic Palaeontology*, doi.org/10.1080/14772019.2018.1426059

- GENERSCH, E., YUE, C., FRIES, I. & DE MIRANDA, J.R. (2006): Detection of deformed wing virus, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities. – *Journal of Invertebrate Pathology* **91**: 61–63.
- GENG, P. et al. (2014): Genetic characterization of a novel iflavirus associated with vomiting disease in the Chinese Oak Silkmoth *Antheraea pernyi*. – *PLoS ONE* **9**: e92107.
- GRIMALDI, D. (2003): A revision of Cretaceous Mantises and their relationships, including new taxa (Insecta: Dictyoptera: Mantodea). – *American Museum Novitates* Number **3412**: 1–47.
- GRIMALDI DA, ENGEL M. & NASCIMBENE P.C. (2002): Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. – *American Museum Novitates* **3361**: 1–72.
- HARWOOD, B.N., DRAPER, I. & KOPIN, A.S. (2014): Targeted inactivation of the rickets receptor in muscle compromises *Drosophila* viability. – *Journal of Experimental Biology* **217**: 4091–4098.
- HESSE-HONEGGER, C. (2001): Heteroptera: The beautiful and the other, or images of a mutating world. – *Scalo*.
- HIYAMA, A. et al. (2013): The Fukushima nuclear accident and the pale grass blue butterfly: evaluating biological effects of long-term low-dose exposures. – *BMC Evolutionary Biology* **13**: 1–25.
- HIYAMA, A. et al. (2012): The biological impacts of the Fukushima nuclear accident on the pale grass blue butterfly. – *Scientific Reports* **2**: 1–10.
- HÖRNIG, M.K., HAUG, J.T. & HAUG, C. (2013): New details of Santanmantis axelrodi and the evolution of the mantodean morphotype. – *Palaeodiversity* **6**: 157–168.
- HÖRNIG, M.K., HAUG, J.T. & HAUG, C. (2017): An exceptionally preserved 110 million years old praying mantis provides new insights into the predatory behaviour of early mantodeans. – *PeerJ* **5**: e3605.
- IQBAL, J. & MUELLER, U. (2007): Virus infection causes specific learning deficits in honeybee foragers. – *Proceedings of the Royal Society B: Biological Sciences* **274**: 1517–1521.
- KANIA, I., WANG, B. & SZWEDO, J. (2015): Dicranoptycha Osten Sacken, 1860 (Diptera, Limoniidae) from the earliest Upper Cretaceous Burmese amber. – *Cretaceous Research* **52**: 522–530.
- KATZOURAKIS, A. (2013): Paleovirology: inferring viral evolution from host genome sequence data. – *Philosophical Transactions of the Royal Society B: Biological Sciences* **368**: 20120493, doi: 10.1098/rstb.2012.0493.
- KOSTRON, B., KALTENHAUSER, U., SEIBEL, B., BRÄUNIG, P. & HONEGGER, H. (1996): Localization of bursicon in CCAP-immunoreactive cells in the thoracic ganglia of the cricket *Gryllus bimaculatus*. – *Journal of Experimental Biology* **199**: 367–377.
- KOČÁREK, P. (2018): *Alienopterella stigmatica* gen. et sp. nov.: the second known species and specimen of Alienoptera extends knowledge about this Cretaceous or-
der (Insecta: Polyneoptera). – *Journal of Systematic Palaeontology*, doi:10.1080/14772019.2018.1440440.
- LANZI, G. et al. (2006): Molecular and biological characterization of deformed wing virus of honeybees (*Apis mellifera* L.). – *Journal of Virology* **80**: 4998–5009.
- LEE, S.W. (2016): Low taxonomic diversity of Blattaria (Insecta) from the Aptian Crato Formation, NE Brazil. – *Geologica Carpathica* **67**: 433–450.
- LIANG, J.H., SHIH, C.K. & REN, D. (2018): New Jurassic predatory cockroaches (Blattaria: Raphidiomimidae) from Daohugou, China and Karatau, Kazakhstan. – *Alcheringa*, doi: 10.1080/03115518.2017.1374460.
- ORUŽINSKÝ, R. & VRŠANSKÝ, P. (2017): Cockroach forewing area and venation variabilities relate. – *Biologia* **72/7**: 814–818, doi: 10.1515/biolog-2017-0090.
- LUO, C.W. et al. (2005): Bursicon, the insect cuticle-hardening hormone, is a heterodimeric cysteine knot protein that activates G protein-coupled receptor LGR2. – *Proceedings of the National Academy of Sciences of the United States of America* **102**: 2820–2825.
- MARTIN, S.K. (2010): Early Jurassic cockroaches (Blattodea) from the Mintaja insect locality, Western Australia. – *Alavesia* **3**: 55–72.
- MILLAN-LEIVA, A. et al. (2012): Genome sequence of SeIV-1, a novel virus from the Iflaviridae family infective to *Spodoptera exigua*. – *Journal of Invertebrate Pathology* **109**: 127–133.
- MURAKAMI, R., SUETSUGU, Y. & NAKASHIMA, N. (2014): Complete genome sequences of two iflaviruses from the brown planthopper, Nilaparvata lugens Artch. – *Archives of Virology* **159**: 585–588.
- PERERA, O.P. et al. (2012): The complete genome sequence of a single-stranded RNA virus from the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). – *Journal of Invertebrate Pathology* **109**: 11–19.
- PODSTRELENÁ, L. & SENDI, H. (2018): Cratovitima Bely, 2007 (Blattaria: Umenocoleidae) recorded in Lebanese and Myanmar ambers. – *Paleontographica Abt A* **310** (3–6): 212–219.
- POINAR, G. JR., POINAR, R. (2005): Fossil evidence of insect pathogens. – *Journal of Invertebrate Pathology* **89**: 243–250.
- ROSS, A.J., MELLISH, C., YORK, P. & CRIGHTON, B. (2010): Burmese Amber. – In: PENNEY D. (ed.): *Biodiversity of fossils in amber from the major world deposits*. – Siri Scientific Press, 208–235.
- SHELLY, L. (2016): Deformed Two Lined Leatherwing. What's That Bug <<http://www.whatsthatbug.com/2016/05/19/deformed-two-lined-leatherwing/>> .
- SHI, G. et al. (2012): Age constraint on Burmese amber based on U-Pb dating of zircons. – *Cretaceous Research* **37**: 155–163.
- SIDORCHUK, E.A. & KHAUSTOV, A.A. (2018): A parasite without host: The first fossil pterygosomatid mite (Acari: Prostigmata: Pterygosomatidae) from French Lower Cretaceous amber. – *Cretaceous Research* **91**: 131–139.

- SILVA, L.S. et al. (2015): Complete genome sequence and structural characterization of a novel iflavirus isolated from *Opsiphanes invirae* (Lepidoptera: Nymphalidae). – *Journal of Invertebrate Pathology* **130**: 136–140.
- ŠMÍDOVÁ L & LEI X (2017): Earliest recorded type-family cockroach was aposematic (Blattaria: Blattidae). *Cretaceous Research* **72**: 189–199.
- TAIRA, W., NOHARA, C., HIYAMA, A. & OTAKI, J.M. (2014): Fukushima's Biological Impacts: The case of the pale grass blue butterfly. – *Journal of Heredity* **105**: 710–722.
- THÉZÉ, J., BÉZIER, A., PERIQUET, G., DREZEN, J.-M. & HERNIOU, E.A. (2011): Paleozoic origin of insect large dsDNA viruses. – *Proceedings of the National Academy of Sciences of the United States of America* **108**: 15932–15935.
- VRŠANSKÝ, P. (2004): Transitional Jurassic/Cretaceous Cockroach assemblage (Insecta, Blattaria) from the Shar-Teg in Mongolia. – *Geologica Carpathica* **55**: 457–468.
- VRŠANSKÝ, P. et al. (2017): Temporary deleterious mass mutations relate to originations of cockroach families. – *Biologia* **72**: 886–912.
- VRŠANSKÝ, P. & BECHLY, G. (2015): New predatory cockroaches (Insecta: Blattaria: Manipulatoridae fam. n.) from the Upper Cretaceous Myanmar amber. – *Geologica Carpathica* **66**: 133–138.
- VRŠANSKÝ, P. & WANG, B. (2017): A new cockroach, with bipectinate antennae, (Blattaria: Olidae fam. nov.) further highlights the differences between the Burmite and other faunas. – *Biologia* **72** (11): 1327–1333.
- WILLIAMS, G.R. et al. (2010): Colony collapse disorder in context. – *BioEssays* **32**: 845–846.
- WHITFIELD, J.B. (2002): Estimating the age of the polydnavirus/braconid wasp symbiosis. – *Proceedings of the National Academy of Sciences of the United States of America* **99**: 7508–7513.
- ZHERIKHIN, V.V. & ROSS, A.J. (2000): A review of the history, geology and age of Burmese amber (Burmite). – *Bulletin of the British Museum (Natural History), Geology* **56**: 3–10.