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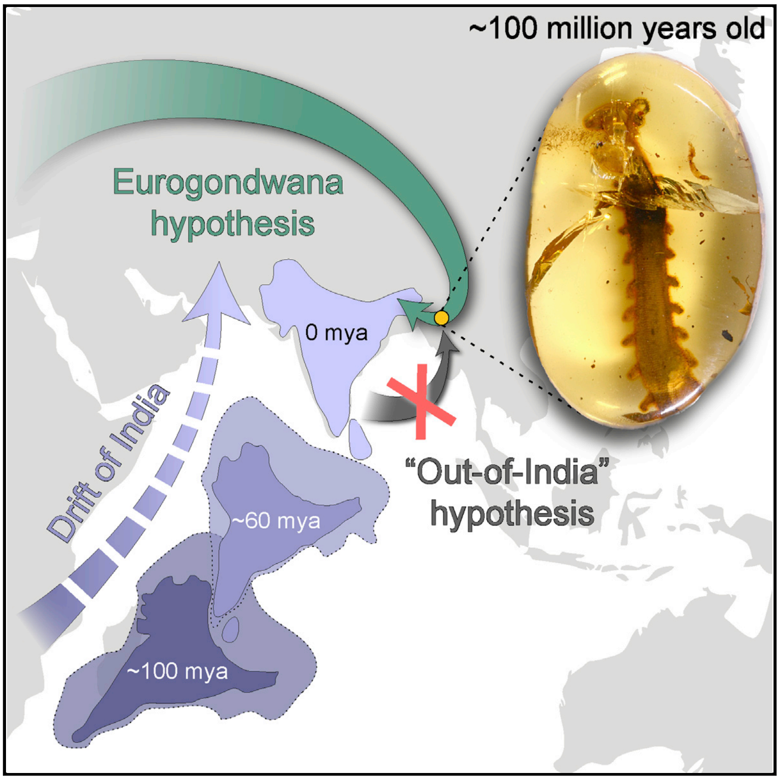
Current Biology

Earliest Onychophoran in Amber Reveals Gondwanan Migration Patterns

Graphical Abstract



Highlights

- The anatomy of the oldest velvet worm in amber is reconstructed using SRμCT
- Gondwanan onychophorans reached Southeast Asia via Europe before the mid-Cretaceous
- The new fossil material elucidates the controversial origin of Indian onychophorans
- Preservation artifacts affected the previous classification of fossil onychophorans

Authors

Ivo de Sena Oliveira, Ming Bai,
Henry Jahn, ..., Jörg U. Hammel,
Weiwei Zhang, Georg Mayer

Correspondence

ivo.oliveira@uni-kassel.de (I.d.S.O.),
baim@ioz.ac.cn (M.B.)

In Brief

Oliveira et al. clarify the evolutionary history of the oldest velvet worm in amber from Myanmar. Their findings suggest that Gondwanan onychophorans reached Southeast Asia prior to continental drift but colonized India only recently after the India-Asia collision, resolving the presence of these Gondwanan organisms in Laurasian-derived regions.

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Earliest Onychophoran in Amber Reveals Gondwanan Migration Patterns

Ivo de Sena Oliveira,[1,2,4,5,*] Ming Bai,[1,4,*] Henry Jahn,[2] Vladimir Gross,[2] Christine Martin,[2] Jörg U. Hammel,[3] Weiwei Zhang,[1] and Georg Mayer[2]
[1]Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Box 92, Beichen West Road 1, Chaoyang District, 100101 Beijing, China
[2]Department of Zoology, Institute of Biology, University of Kassel, Heinrich-Plett-Straße 40, 34132 Kassel, Germany
[3]Institute of Materials Research, Helmholtz-Zentrum Geesthacht, Max-Planck-Str. 1, 21502 Geesthacht, Germany
[4]Co-first author
[5]Lead Contact
*Correspondence: ivo.oliveira@uni-kassel.de (I.d.S.O.), baim@ioz.ac.cn (M.B.)
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SUMMARY

The anomalous occurrence of supposedly Gondwanan taxa in Laurasian-derived regions remains an intriguing chapter of paleobiogeographical history. Representatives of Peripatidae, a major subgroup of velvet worms (Onychophora), show a disjointed distribution in the neotropics, tropical Africa, and Southeast Asia, the latter being the only landmass previously associated with Laurasia [1, 2]. The arrival of these animals in Southeast Asia is explained by two alternative, albeit not mutually exclusive, hypotheses: an early migration via Europe before continental drift (Eurogondwana hypothesis) or transportation via insular India during the Cretaceous and Paleogene ("out-of-India" hypothesis) [3–6]. The latter hypothesis is based on a single extant species of Peripatidae, *Typhloperipatus williamsoni*, in India. †*Cretoperipatus burmiticus* from Myanmar is the oldest fossil onychophoran found in amber [7], dating to sometime between the two proposed scenarios, and hence crucial for clarifying how Gondwanan lineages of these low-vagility animals reached Southeast Asia (see also Supplemental Information). Based on the anatomical reconstruction of †*C. burmiticus* using synchrotron radiation-based X-ray microtomography (SRμCT) and comparisons with extant taxa, we resolved this fossil species within Onychophora, particularly within Peripatidae, with *T. williamsoni* as its closest extant relative. This suggests that an early Eurogondwanan migration of peripatids was the most likely event, as Burmese amber is too old to be compatible with the out-of-India hypothesis. Moreover, peripatids probably colonized India only recently from Myanmar, refuting the putative Gondwanan relict status of Indian onychophorans. Finally, preservation artifacts identified in the novel amber material might have a major impact on studies of onychophoran stem and/or crown groups.

RESULTS

†*Cretoperipatus burmiticus* described from Burmese amber [7] is the oldest and only known Mesozoic amber-embedded specimen of Onychophora, dating back to the earliest Cenomanian (~100 million years ago [mya]) according to paleontological data and *U–Pb* dating of zircons from the amber matrix [8, 9]. However, the poor preservation of the single specimen previously found precluded major conclusions about this species. Herein, light microscopy and synchrotron radiation-based X-ray microtomography (SRμCT) applied to three newly discovered specimens of †*C. burmiticus* revealed numerous hitherto unknown features of this fossil species (Figures 2 and 3; see Supplemental Information for full morphological characterization), including a remarkable similarity to extant Southeast Asian peripatids, as demonstrated by a morphological comparison with an undescribed species of *Eoperipatus* from Thailand (Figures S1 and S2).

Systematic Paleontology

Onychophora Guilding, 1853 [10].
Peripatidae Evans, 1901 [11].
†*Cretoperipatus burmiticus* Engel and Grimaldi, 2002 [7] (Figures 1, 2, 3, and S1–S4).
LSID urn:lsid:zoobank.org:act:12E4F8E3-6E0D-41E8-8A13-B36369FCC90E (http://zoobank.org/NomenclaturalActs/12E4F8E3-6E0D-41E8-8A13-B36369FCC90E).

Referred Material

Topotypes in yellow Burmese amber (Figure 1); BU-001467 (nearly complete albeit suboptimally preserved adult; undefined sex); BU-001468 (exceptionally preserved anterior half of an adult; undefined sex) and ZZZ0066 (complete albeit suboptimally preserved juvenile; undefined sex).

Emended Diagnosis

†*Cretoperipatus burmiticus* exhibits the following unique combination of characters: basal piece of dorsal primary papillae composed of two lateral and five anterior or posterior scale ranks (Figure 2E); apical piece asymmetrical, with three anterior and two posterior scale ranks (Figure 2E); distal- and proximal-most spinous pads of the legs shorter than the two in between, the proximal-most one being thinner than the others (Figure 3C); nephridial tubercle located in the third pad, splitting it into two unequal parts

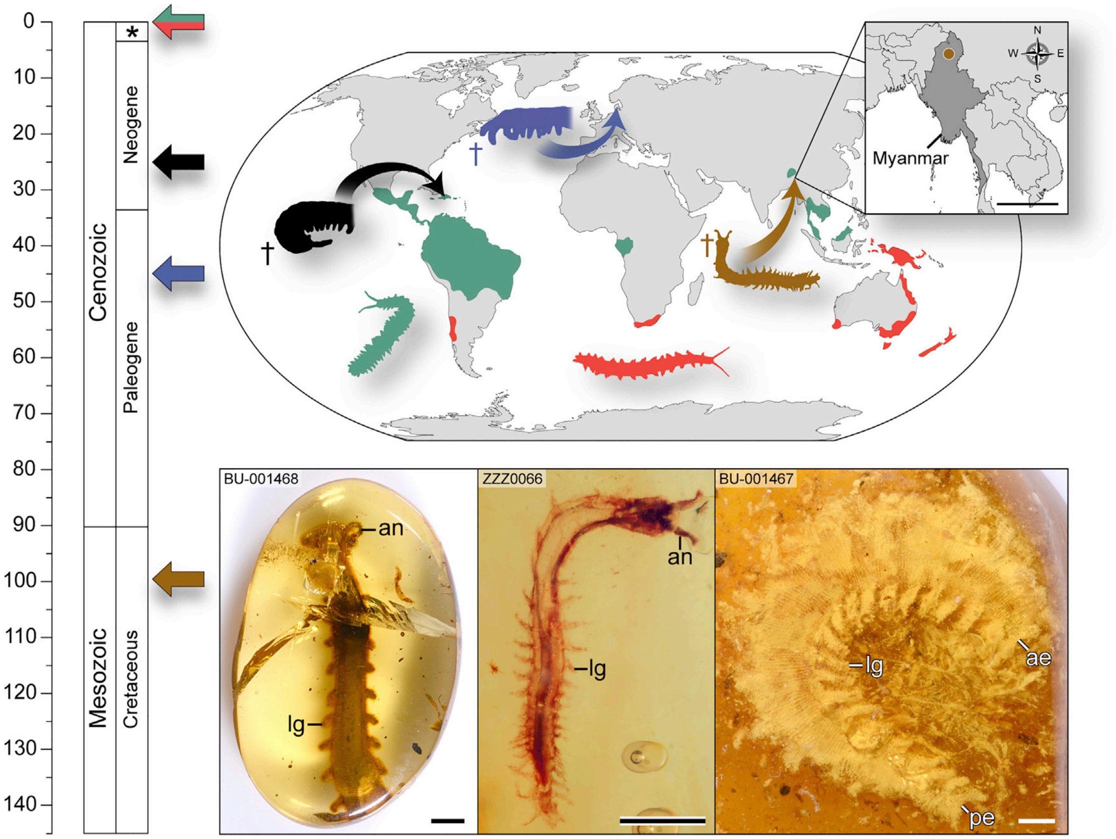

Figure 1. Spatiotemporal Distribution of Extant and Amber-Embedded Onychophorans

Present-day distribution of Peripatidae is represented in green and of Peripatopsidae in red. The localities of known amber-embedded onychophorans (†) are indicated in black for †*Tertiapatus dominicanus* (Dominican amber), in purplish blue for †*Succinipatopsis balticus* (Baltic amber), and in brown for †*Cretoperipatus burmiticus* (Burmese amber). The temporal distribution of fossil and extant onychophorans is indicated on the left side by arrows of the corresponding color. Asterisk indicates the Quaternary period, to which the present day belongs. Map inset indicates the locality in Myanmar where the studied specimens (light micrographs) were found. Abbreviations are as follows: ae, anterior body end; an, antenna; lg, leg; pe, posterior body end. Scale bars represent 1.5 mm.

(arrowhead in Figure 3D); apical collar of type I crater-shaped papillae bearing eight scales (Figure 3F); ventral fields of modified scales [12] or interpedal structures [13, 14] absent.

Differential Diagnosis

Herein, †*C. burmiticus* is resolved within Peripatidae based on the following autapomorphies of this subgroup [14–17]: primary dermal papillae subdivided into basal and apical pieces (Figure 2E), spindle-shaped sensilla forming the antennal sensory fields (Figure 3A), presence of a diastema and a diastemal membrane in the inner jaw blade (Figure S1D), and genital pad situated between the penultimate legs (Figure S2A). Within Peripatidae, the presence of one anterior and one posterior distal foot papilla (Figure 3B) classifies †*C. burmiticus* among representatives of the Southeast Asian Peripatidae [11, 12, 14, 18], i.e., the extant taxa *Eoperipatus* and *Typhloperipatus*, thus differing from all extant neotropical forms—a monophyletic group [3] herein named Neopatida—as well as from the tropical African *Mesoperipatus*, which show at least three distal foot papillae [14]. Different from *Eoperipatus* spp., †*C. burmiticus* lacks ventral fields of modified scales (present at least in *E. totoro* and *Eoperipatus* sp.; see [12]) and shows the nephridial tubercle in the third spinous pad, while this structure appears further proximally (Figure S2C′) in *Eoperipatus* [11, 14, 19]. Although the position of the nephridial tubercle in †*C. burmiticus* is shared with the monotypic *Typhloperipatus* from India [18, 20], these species are clearly distinct because well-developed eyes are present in †*C. burmiticus* (Figure 2B and Movie S1), but not in *T. williamsoni* [14, 18]. Further taxonomically relevant characters of the Southeast Asian peripatids [11, 12, 14, 18] could not be compared, as neither internal structures nor the posterior end of a male have been preserved in the fossil material studied.

DISCUSSION

Knowledge of the evolutionary history of the only three described species of amber-embedded velvet worms [7, 21] has been stymied by suboptimal preservation of the few specimens available together with the limited imaging methods previously applied. In this respect, the newly discovered material of †*C. burmiticus* [7, 8] is relevant not only because it enabled us to reconstruct in detail the anatomy of an ∼100 million-year-old fossil species (Figures S4A–S4D) and resolve its uncertain [2] position within Onychophora but also because it provides new information to

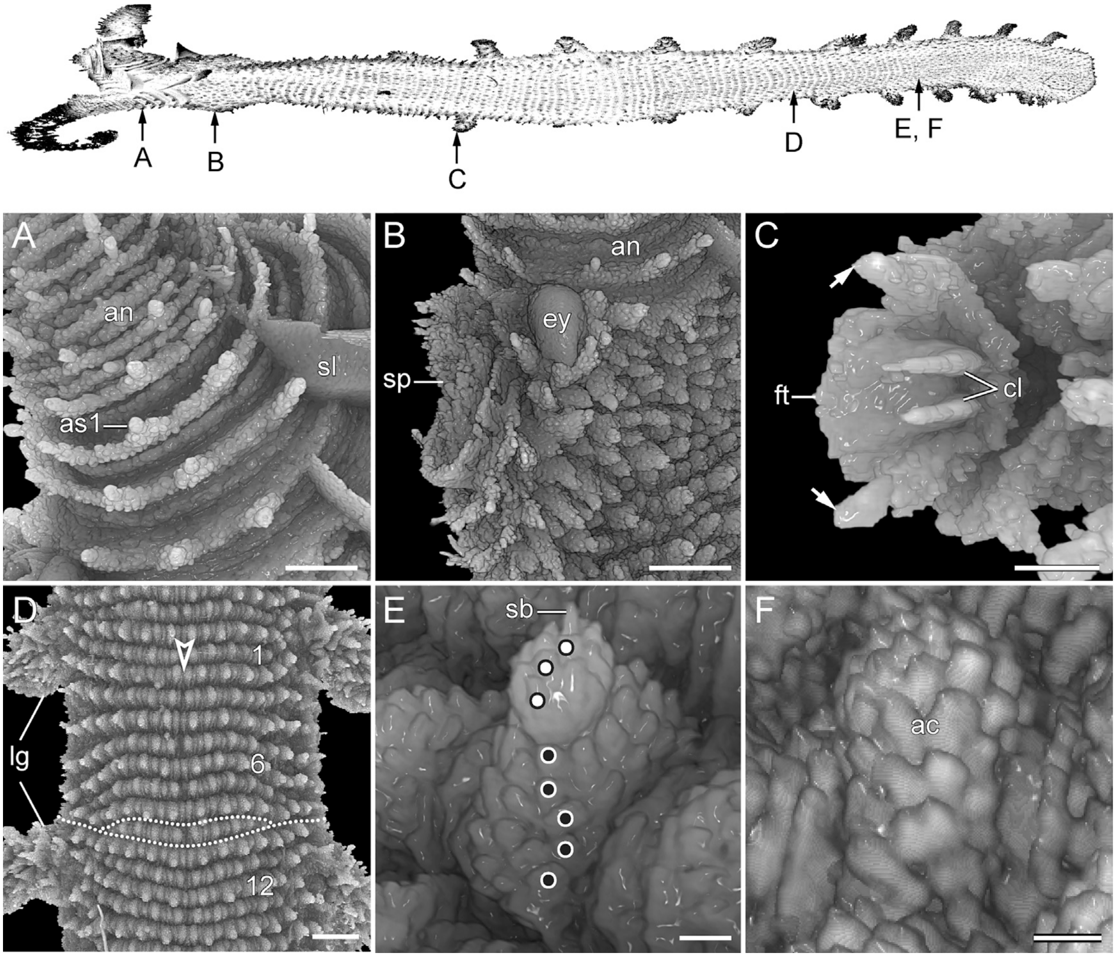

Figure 2. Dorsal Anatomy of †*Cretoperipatus burmiticus*

(A–F) Simplified overview and detailed volume rendering of BU-001468. Anterior is up in (A)–(D) and down in (E) and (F).

(A) Basis of the left antenna.

(B) Left eye and retracted slime papilla.

(C) Foot in distal view. Arrows indicate distal foot papillae.

(D) Overview of the dorsal integument. Arrowhead points to the dorsomedian furrow. Segmental plicae are numbered. Dotted lines delineate a shorter plica.

(E) A dorsal primary papilla. Black dots with white circle and white dots with black circle indicate the number of scale ranks in the basal and apical pieces, respectively.

(F) A dorsal accessory papilla.

Abbreviations are as follows: ac, accessory papilla; an, antenna; as1, type I sensillum; cl, claw; ey, eye; ft, foot; lg, leg; sb, sensorial bristle; sl, slime; sp, slime papilla. Scale bars represent 100 μm in (A), 300 μm in (B) and (D), 15 μm in (C) and (F), and 25 μm in (E). See also Figures S1–S4 and Movies S1, S2, and S3.

explore unclear evolutionary and paleobiogeographical aspects long associated with this ancient animal group.

First, our data revealed that, although onychophorans are soft-bodied organisms known to be highly prone to a fast decay [22], preservation artifacts had not been considered carefully in previous studies of amber-embedded velvet worms and may have led to the misinterpretation of important anatomical features. The main example of this is the controversial absence of feet and claws, presented as diagnostic characters, in Baltic and Dominican fossil onychophorans [7, 21]. Recent decay experiments showed that the typical onychophoran foot [16, 23] may start decomposing immediately after death [22], and since a fragile membrane is the only connection between the claws and the foot [16], its decay may result in the claws detaching. Our findings demonstrate that claws indeed detach from the decaying specimen, often being found dispersed in the amber sample, sometimes far from the preserved specimen, which in turn may not exhibit any remaining feet or claws. The presence of stacked cuticular elements [24] in the detached claws found in our material (arrows in Figure S1F) suggests that they belonged to the original specimen rather than to a putative exuvium, as only the outer-most cuticular layer of the jaws and claws are molted [25]. Hence, it is likely that these structures had detached from the Baltic and Dominican specimens [21, 26] and went unnoticed or even became completely lost due to trimming of the amber for optimal visualization of the specimen. If this holds true, the classification and suggested position of these amber fossils within the total group Onychophora [27–29] should be revised.

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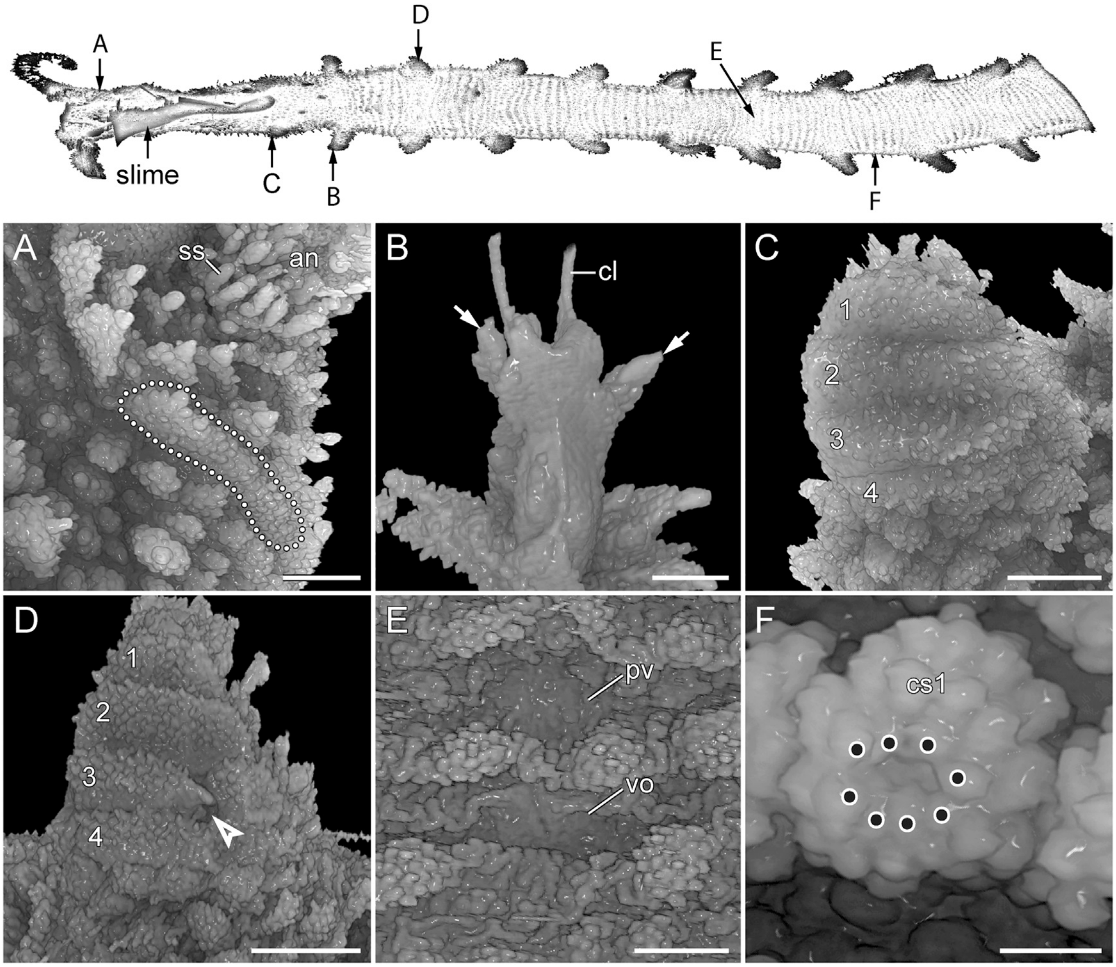

Figure 3. Ventral Anatomy of †*Cretoperipatus burmiticus*

(A–F) Simplified overview and detailed volume rendering of BU-001468. Anterior is up in (A), (E), and (F); distal is up in (B), (C), and (D).

(A) Basis of the left antenna. Dotted line delineates the frontal organ.

(B) Foot in ventral view. Arrows point to distal foot papillae.

(C) Second right leg. Spinous pads are numbered.

(D) Fourth left leg. Spinous pads are numbered. Arrowhead points to the nephridial tubercle.

(E) Preventral and ventral organs.

(F) A type I crater-shaped papilla. Black dots with white circles indicate scales forming the apical collar.

Abbreviations are as follows: an, antenna; cl, claw; cs1, type I crater-shaped papilla; pv, preventral organ; ss, spindle-shaped sensillum; vo, ventral organ. Scale bars represent 100 μm in (A) and (C), 10 μm in (B), 200 μm in (D), 50 μm in (E), and 20 μm in (F). See also Figures S1–S4 and Movies S1, S2, and S3.

This might also extend to a recent study of the Carboniferous fossil †*Helenodora inopinata* [29], the anatomical interpretation of which, in our view, should be carefully reconsidered. The classification of this species within either the crown or stem group Onychophora remains debatable largely because of uncertainties regarding the presence of typical onychophoran features, such as claws and slime papillae [24, 29–33]. These structures had previously been identified in †*H. inopinata* [32, 33] but interpreted as taphonomic artifacts in a recent study [29], which suggested that both claws and slime papillae were in fact absent in this species. However, Murdock et al. [29] identified “small dark patches” in several specimens—comparable in size, shape, and composition to those structures previously regarded as claws—but did not interpret them as claws because they were inconsistently distributed, sometimes outside the limits of the fossil. Considering that we also confirmed this phenomenon in †*C. burmiticus*, these dark patches in †*H. inopinata* could reasonably be interpreted as claws that were separated from the decaying specimen prior to or during fossilization.

We also observed that the slime papillae, unlike the legs, are highly retractile and could hardly be distinguished from the surrounding integument of †*C. burmiticus*, even using powerful imaging techniques (Movie S1). This raises the question of whether the slime papillae were indeed absent in †*H. inopinata* [29], or whether the preservation of the analyzed specimens simply prevented their observation. Since it is difficult to decide between these two possibilities, and since claws could have been present in †*H. inopinata*, the recent assumption that this species belongs

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to the onychophoran stem group [29] should be considered with caution.

Preservation artifacts and methodological constraints might also have affected the only previous study [7] of †*C. burmiticus*, as neither the antero-posterior orientation of the single specimen found nor the classification of this fossil species within Onychophora could be resolved unambiguously [2]. Herein, we were able to close both of these gaps based on specific morphological correspondences found between our data on †*C. burmiticus*, those originally presented for this fossil species, and those available for other extant onychophorans (see Supplemental Information for further details). Using the exclusive anterior position of the nephridial tubercle on the fourth and fifth leg pairs and the typical reduction in the number of spinous pads in posterior leg pairs as morphological landmarks [12–14], we suggest that the incomplete holotype of †*C. burmiticus* was originally misinterpreted as the anterior, while it in fact represents the posterior half of the animal. In addition, our findings also resolve †*C. burmiticus* unambiguously within Peripatidae and classify it among the Southeast Asian peripatids based on the unique number and arrangement of distal foot papillae in representatives of this clade.

Assessing the position of †*C. burmiticus* within the Southeast Asian Peripatidae, however, is more difficult due to the limited information currently available from most taxa of this group. Nevertheless, a literature survey suggests that the Indian *T. williamsoni* is morphologically more similar to †*C. burmiticus* [12, 18, 20] than the latter is to any representative of *Eoperipatus* from Thailand, Vietnam, and/or Malaysia. Most important is the unique combination of characters shared by *T. williamsoni* and †*C. burmiticus* on their fourth and fifth leg pairs, i.e., four complete spinous pads, a nephridial tubercle situated in the third pad, and only one anterior and one posterior distal foot papilla (Figure S4D). On the other hand, no taxonomically informative character supports a closer relationship between †*C. burmiticus* and representatives of *Eoperipatus*.

On this basis, two evolutionary scenarios are possible, neither of which can be unambiguously ruled out: †*C. burmiticus* either forms, together with *Typhloperipatus*, a sister clade to *Eoperipatus*, or it represents a taxon basal to all extant Southeast Asian Peripatidae. We favor the first hypothesis (Figure 4A) because the position of the nephridial tubercle observed in †*C. burmiticus* and *Typhloperipatus* is uncommon within Peripatidae [14], while in *Eoperipatus* spp. it is either the same as in nearly all remaining peripatid species [12, 14] or further proximal [11, 19]. Therefore, it is more parsimonious to assume that the ancestral state of this character was originally retained in *Eoperipatus* and modified in the †*Cretoperipatus* + *Typhloperipatus* lineage.

The presented evolutionary scenario, however, has major implications for the paleobiogeographical history of Onychophora. The confirmation of peripatids inhabiting Southeast Asia already during the Cretaceous, a time when India was an island completely isolated from any continental landmasses (Figure 4B), refutes, at least for Onychophora, the "out-of-India" hypothesis [6], according to which India acted as a raft bringing Gondwanan lineages to Southeast Asia. Alternatively, the recent paleogeographical Eurogondwana model [5], according to which Gondwanan lineages colonized Laurasia prior to continental drift via the Apulia microplate and subsequently went extinct in the Northern Hemisphere, seems to be the most likely scenario accounting for the presence of Peripatidae in Southeast Asia.

Even assuming a Eurogondwanan migration, onychophorans, like other animals and plants [6], could well have spread to India prior to the subcontinent's split from Gondwana approximately 120 mya [1]. As such, the biogeographical importance of the only piece of evidence from present-day India, *Typhloperipatus williamsoni*, cannot be understated. Because this species is restricted to a very small region in the easternmost part of India [18, 20], it could have been subjected to the long isolation period corresponding to India's northward drift toward [34, 35] and collision with Southeast Asia approximately 25 mya [1] and could still represent a Gondwanan relict population in this region.

The evolutionary position of †*C. burmiticus* as sister to *T. williamsoni* rather than to *Eoperipatus* species (Figure 4A), however, speaks against this putative Gondwanan relict status of *Typhloperipatus* in India. If one assumes that a peripatid population, in this case *Typhloperipatus*, persisted in India from its split with Gondwana (∼120 mya) until present day, one would also have to assume that many morphological features currently shared by Southeast Asian peripatids [14, 18], including †*C. burmiticus*, evolved convergently in the Indian *Typhloperipatus*. Instead, it is more parsimonious to assume that a post-Gondwanan colonization of India by peripatids occurred after the so-called India-Asia collision [35], thus explaining the morphological similarities among all Southeast Asian peripatids.

Three additional arguments speak in favor of a post-Gondwanan colonization of India by peripatids (see Supplemental Information for further details). First, India was previously associated with landmasses corresponding to present-day Australia, Antarctica, and South Africa [1, 35] (Figure 4B). Apart from Antarctica, which lacks living onychophorans, the other two areas are colonized exclusively by Peripatopsidae [2, 15, 36–38]; therefore, it is reasonable to assume that, if any Gondwanan-derived population of Onychophora ever persisted in India, it would also be composed of peripatopsids. Second, insular India had a hostile paleoclimate during the Cretaceous [39] and underwent drastic geological events [34, 40], suggesting that a viable population of Onychophora would be unlikely to survive in India during this period. Finally, onychophorans have never been reported from Madagascar, even though this landmass shares a similar paleogeographical history with India [1] (Figure 4B). In the event that a Gondwanan relict population of Onychophora, irrespective of whether it belonged to Peripatidae or Peripatopsidae, had persisted in India and endured geological and climate events of the Cretaceous, one would expect the same to have occurred in Madagascar.

Despite the evidence for Eurogondwanan migration and post-Gondwanan colonization of India by peripatids, it is still difficult to determine precisely when these animals underwent the dispersal and extinction events that shaped their current distribution. However, it is clear that representatives of Peripatidae reached Southeast Asia prior to the earliest Cenomanian (∼100 mya), to which †*C. burmiticus* has been dated [8]. Fossil record indicates that onychophorans indeed inhabited the Northern Hemisphere during the Paleozoic [3]; thus, their current absence from Europe, as well as the disjoint distribution pattern of Peripatidae, can be logically explained by extinction.

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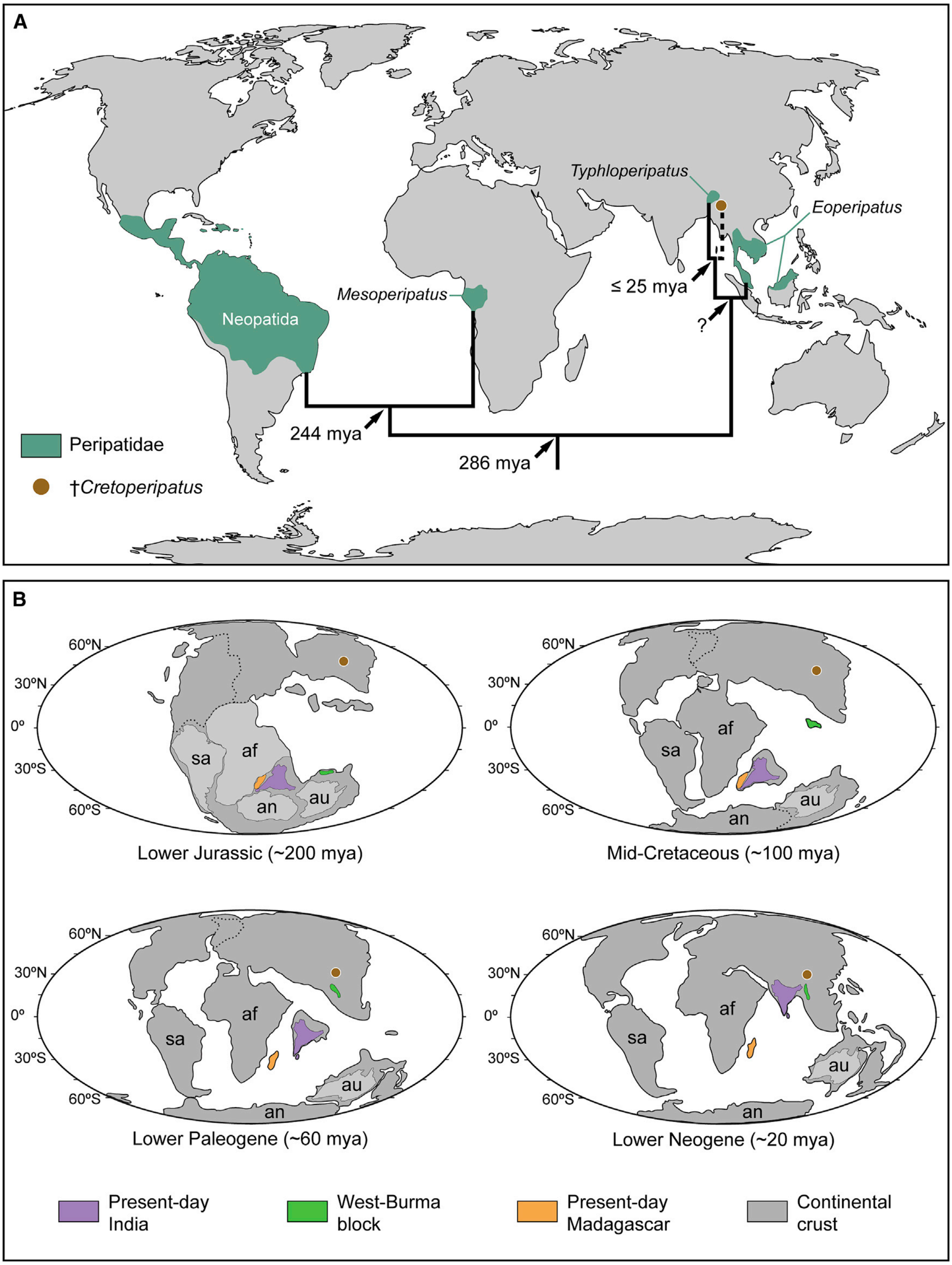

Figure 4. Phylogenetic Position and Paleogeographic History of †*Cretoperipatus burmiticus*

Brown dots indicate the estimated position of the future amber deposit of †*C. burmiticus*.

(A) Phylogenetic relationships and divergence time of the main lineages within Peripatidae. Dashed line indicates the most likely phylogenetic position of †*C. burmiticus*. Arrows indicate divergence times estimated herein and in a previous molecular study [3].

(B) Paleogeographic history of areas currently associated with the type locality of †*C. burmiticus*. Maps reconstructed after Seton et al. [1].

Abbreviations are as follows: af, Africa; an, Antarctica; au, Australia; sa, South America.

We estimate that the colonization of India by Peripatidae could not have occurred earlier than the latest Oligocene (~25 mya), which marks the second of a two-stage collision of India with Asia [35]. This is the first time that a terrestrial connection had been established between India and Asia and the earliest period when peripatids were able to disperse into India—most likely from present-day Myanmar given the morphological resemblance between †*C. burmiticus* and *T. williamsoni*. To date, extant onychophorans have never been reported from Myanmar, although living representatives of Peripatidae occur in neighboring countries [12, 14]. Since this may simply represent a sampling bias, an eventual discovery of living onychophorans in Myanmar could bring more clarity to the scenario presented herein and shed light on the paleobiogeographical history of many other low-vagility invertebrate taxa, which also show purportedly Gondwanan lineages occurring in Laurasian-derived territories.

EXPERIMENTAL PROCEDURES

Specimens in amber were obtained from a fossiliferous deposit in the Noije Bum, near Tanai Village (26°21′33.41″N, 96°43′11.88″E), Hukawng Valley, Myanmar [41–43]. Specimens of *Eoperipatus* sp. used for comparative purposes were obtained and maintained in culture as described previously [14]. Amber pieces were documented using a Zeiss Axio Zoom.V16 stereomicroscope (Carl Zeiss) and scanned with a MicroXCT-400 (Carl Zeiss X-ray Microscopy). SRμCT was carried out at the Shanghai Synchrotron Radiation Facility, the Beijing Synchrotron Radiation Facility, and the Deutsches Elektronen Synchrotron (DESY). Dataset reconstruction, image stack treatment, and volume rendering were performed as previously described [25, 44, 45]. Scanning electron microscopy of *Eoperipatus* sp. was conducted as described elsewhere [12, 14]. Terminology of morphological structures follows Oliveira et al. [12, 14, 17]. A complete morphological description of the material is available in the Supplemental Information.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.07.023.

AUTHOR CONTRIBUTIONS

Conceptualization, I.d.S.O. and G.M.; Investigation, I.d.S.O., M.B., G.M., H.J., C.M., V.G., and J.U.H.; Resources, I.d.S.O., G.M., M.B., and W.Z.; Writing – Original Draft, I.d.S.O. and G.M.; Writing – Review & Editing, I.d.S.O., M.B., W.Z., G.M., H.J., C.M., V.G., and J.U.H.; Visualization, I.d.S.O., H.J., J.U.H., and G.M.; Funding Acquisition, G.M., J.U.H., and M.B.; Supervision, G.M. and M.B.

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Current Biology, Volume *26*

Supplemental Information

Earliest Onychophoran in Amber Reveals Gondwanan Migration Patterns

Ivo de Sena Oliveira, Ming Bai, Henry Jahn, Vladimir Gross, Christine Martin, Jörg U. Hammel, Weiwei Zhang, and Georg Mayer

SUPPLEMENTAL FIGURES

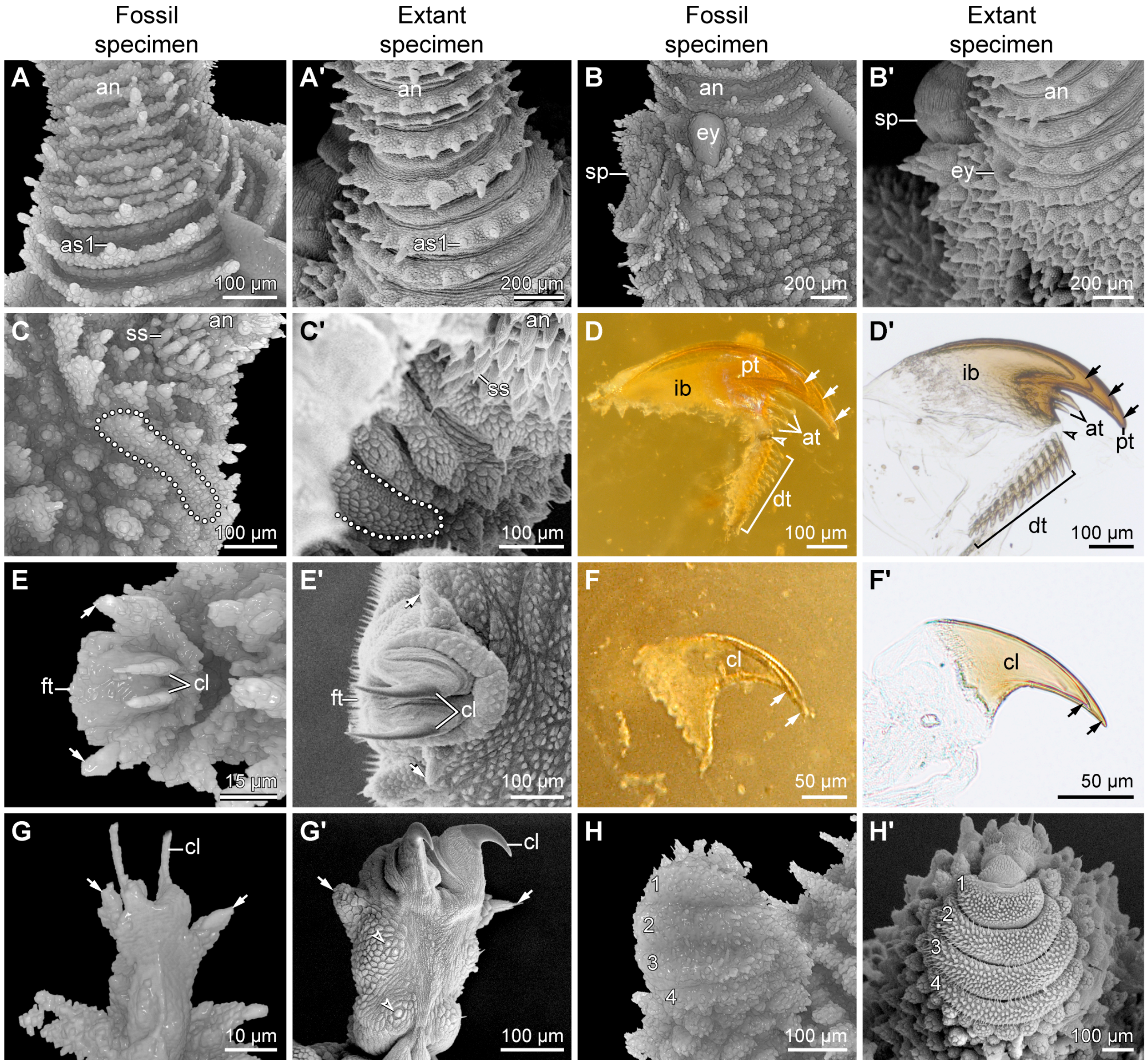

Figure S1. Morphological comparison between †*C. burmiticus* and an extant Southeast Asian peripatid. Related to Figures 2 and 3. (A–H) Volume rendering of BU-001468 (A–C, E, G, H) and light micrographs of BU-001467 (D, F). (A'–H') Scanning electron micrographs (A'–C', E', G', H') and light microscopy (D', F') from corresponding regions in an undescribed species of *Eoperipatus* from Thailand. Anterior is up in A–E and right in G and H; distal is right in F. Arrows point to the stacked elements of jaws and claws in D and F and to distal foot papillae in E and G. (A) Dorsal basis of the left antenna. (B) Left eye and slime papilla. (C) Ventral basis of the left antenna. Dotted line delineates the frontal organ. (D) Inner jaw blade. Arrowhead points to the diastema enclosing the diastemal membrane. (E) Foot in distal view. (F) A claw. (G) Foot in ventral view. (H) A leg in ventral view. Spinous pads are numbered. Abbreviations: an, antenna; as1, type I sensillum; at, accessory teeth; cl, claw; dt, denticle blade; ey, eye; ft, foot; ib, inner jaw blade; pt, principal tooth; sp, slime papilla; ss, spindle-shaped sensillum.

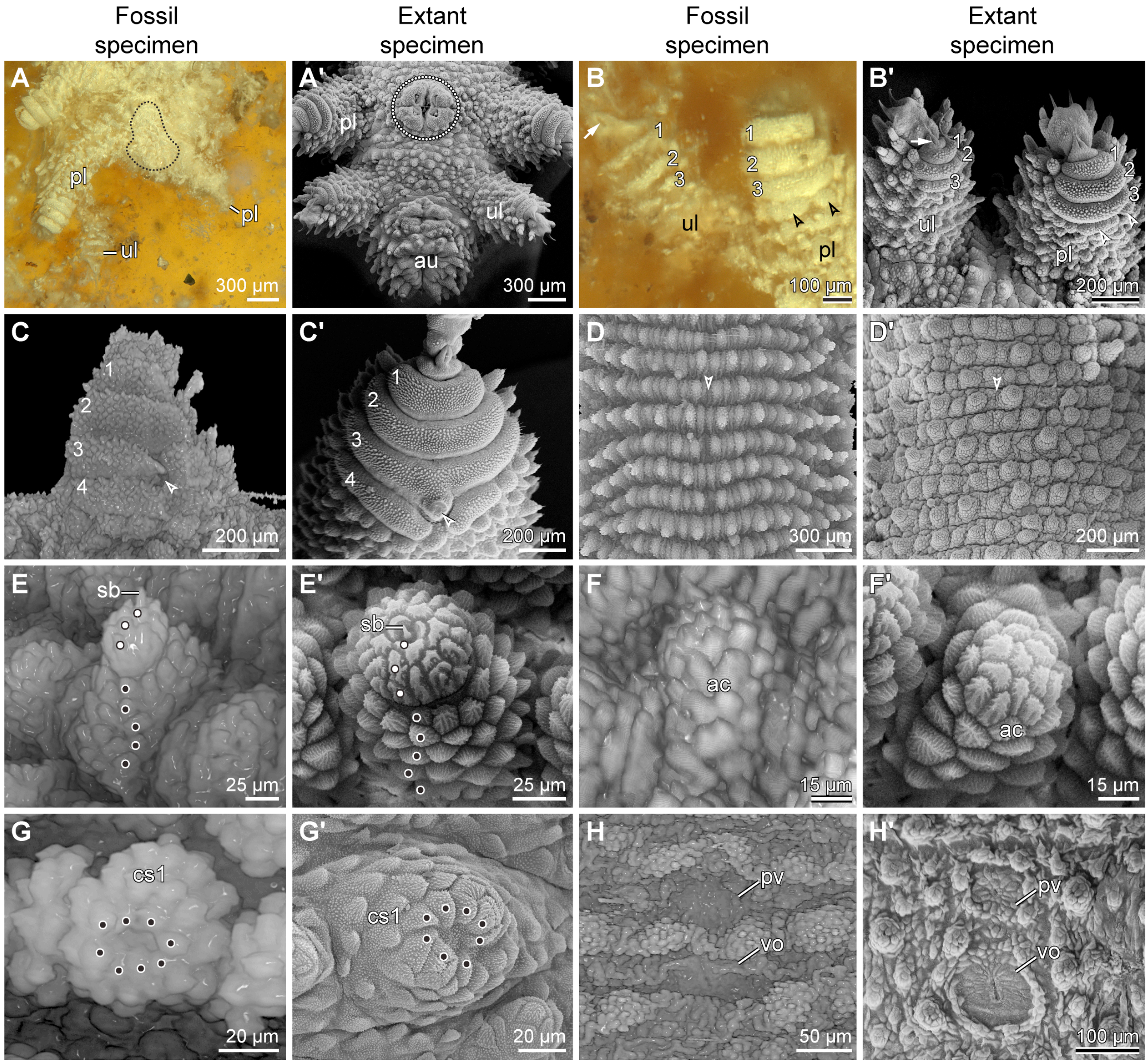

Figure S2. Morphological comparison between †*C. burmiticus* and an extant Southeast Asian peripatid. Related to Figures 2 and 3. (A–H) light micrographs of BU-001467 (A, B) and volume rendering of BU-001468 (C–H). (A'–H') Scanning electron micrographs from corresponding regions in an undescribed species of *Eoperipatus* from Thailand. Anterior is up in A, D and H, right in B and C, and down in E–G. Spinous pads are numbered in B and C. Arrowhead points to the fragmented fourth pad in B, the nephridial tubercle in C, and the dorsomedian furrow in D. (A) Posterior body-end in ventral view. Dotted line delineates the genital opening. (B) Penultimate and last legs. Arrow indicates the foot bridge. (C) Fourth left leg. (D) Overview of the dorsal integument. (E) A dorsal primary papilla. Black dots with white circle and white dots with black circle indicate the number of scale ranks in the basal and apical pieces, respectively. (F) A dorsal accessory papilla. (G) A type-I crater-shaped papilla. Black dots with white circles indicate scales forming the apical collar. (H) Preventral and ventral organs. Abbreviations: ac, accessory papilla; au, anus; cs1, type I crater-shaped papilla; pl, penultimate leg; pv, preventral organ; sb, sensorial bristle; ul, last leg; vo, ventral organ.

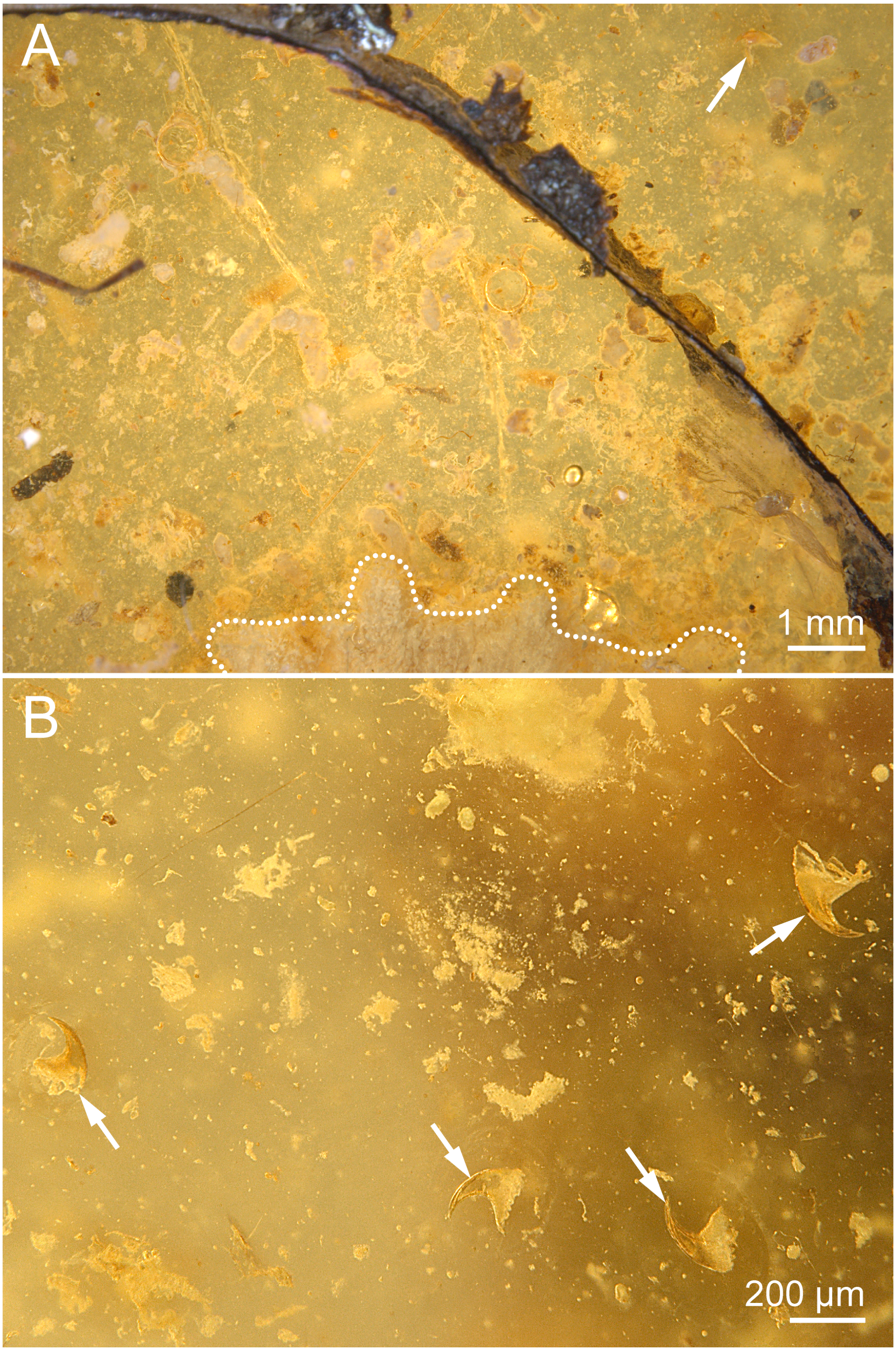

Figure S3. Dispersed jaw fragment and claws in the amber piece studied. Related to Figures 2 and 3. Light micrographs of BU-001467. Arrows point to a jaw fragment (in A) and claws (in B) found relatively far from the specimen (dotted line in A), which does not preserve these structures in their corresponding anatomical position any longer.

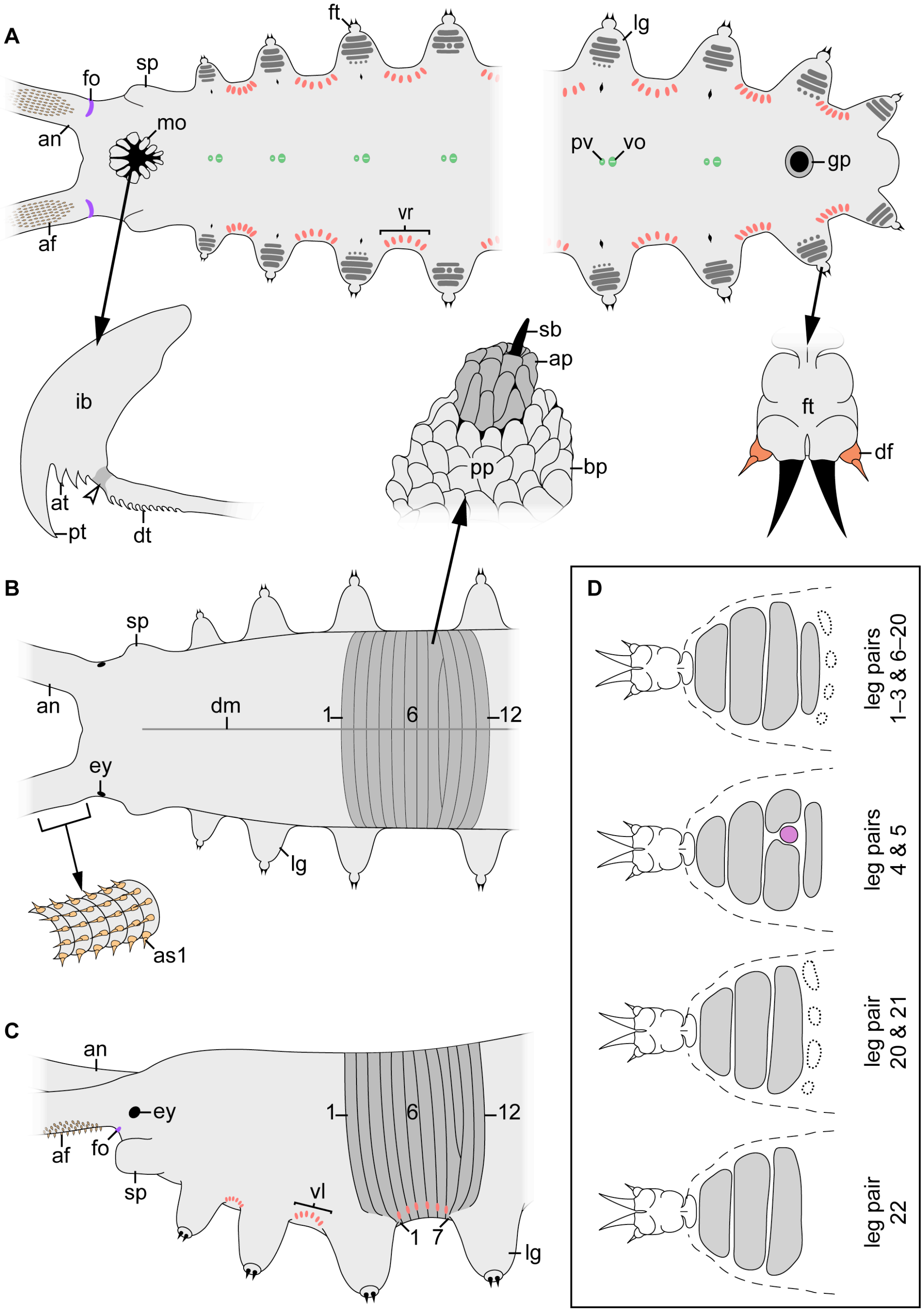

Figure S4. Summary of anatomical features identified in †*Cretoperipatus burmiticus*. Related to Figures 2 and 3. Simplified diagrams. Anterior is left in A–C and up in D. Insets (arrows) show details of taxonomically informative structures. (A) Ventral body region. Arrowhead points to the diastema with a diastemal membrane of the inner jaw blade. (B) Dorsal body region. Segmental plicae are numbered. (C) Lateral body region. Type II crater-shaped papillae are numbered. (D) Variation of the leg structure along the antero-posterior body axis. Dotted lines indicate fragmented spinous pads. Abbreviations: af, antennal sensory field with spindle-shaped sensilla; an, antenna; ap, apical piece; as1, type I sensillum ; at, accessory teeth; bp, basal piece; df, distal foot papilla; dm, dorsomedian furrow; dt, denticle blade; ey, eye; fo, frontal organ; ft, foot; gp, genital pad; ib, inner jaw blade; lg, leg; mo, mouth; pp, primary papilla; pt, primary tooth; pv, preventral organ; sb, sensory bristle; sp, slime papilla; vl, ventrolateral row of crater-shaped papillae; vo, ventral organ; vr, ventral row of crater-shaped papillae.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Material studied

Three specimens of the onychophoran species †*Cretoperipatus burmiticus* Engel & Grimaldi, 2002 preserved in yellow-colored Burmese amber were obtained from a rich fossiliferous deposit [S1-3] in the Noije Bum, near Tanai Village (26°21'33.41"N, 96°43'11.88"E), Hukawng Valley, Myanmar (Figure 1). Previous dating analyses suggest that amber from this region dates to the earliest Cenomanian, approximately 100 mya [S4, S5]. Further details on the excavation history and stratigraphy of Burmese amber have been provided previously elsewhere [S4]. The largest specimen studied (~26 mm) appears nearly complete, albeit poorly preserved, and is accompanied by several plant and arthropod fragments in the amber piece (BU-001467). The medium-sized specimen (~13 mm) consists of an exceptionally preserved anterior half (BU-001468), whereas the smallest specimen (~5 mm) represents a complete, albeit poorly preserved, juvenile (ZZZ0066). The amber pieces containing the latter two specimens do not show any conspicuous paleobiota. The specimens studied have been loaned to the Institute of Zoology, Chinese Academy of Sciences (IZAS) and are available for further investigation by contacting MB or WZ. In the future, BU-001467 and BU-001468 will be deposited in the Three Gorges Entomological Museum, Chongqing, China and ZZZ0066 will be placed in the private collection of Zezhao Zheng (PCZZ; 10-3-202, Yixiangyuan, Renmin west road, Zhangdian District, Zibo, Shandong Province, China). Specimens of *Eoperipatus* sp. – an extant undescribed species from Thailand used for comparative purposes – were obtained and maintained in culture as described previously [S6].

Morphological analyses and image processing

The amber pieces containing the onychophoran fossils have been trimmed and polished for an optimal view of the specimens and documented using a stereomicroscope Zeiss Axio Zoom.V16 (Carl Zeiss, Jena, Germany) equipped with a digital camera (Zeiss Axiocam 503 color). All specimens were initially scanned with a MicroXCT-400 (Carl Zeiss X-ray Microscopy, Inc., Pleasanton, USA) at the Institute of Zoology, Chinese Academy of Sciences. Non-invasive propagation phase contrast synchrotron radiation x-ray microtomography (PPC-SRµCT) has been carried out on the beamline 13W of the Shanghai Synchrotron Radiation Facility (SSRF) and on the beamline 3W1A of the Beijing Synchrotron Radiation Facility (BSRF). Scans of BU-001467 and ZZZ0066 did not retrieve valuable morphological information because of the similar x-ray density of the amber piece and the preserved specimen. The amber piece containing BU-001468 has been further mounted onto a standard sample holder and analyzed using a high-resolution synchrotron radiation-based X-ray microtomography (=SRµCT) operated by Helmholtz-Zentrum-Geesthacht at Deutsches Elektronen Synchrotron (DESY, Hamburg, Germany). This specimen was scanned in 8 subsequent tiers ($\Delta Z = 2.2$ mm) at 23 keV in the beamline P05 [S7] of the DESY storage-ring PETRA III. Each tier yielded 1200 equally spaced projections between 0 and π. Reconstruction of the datasets was performed using the algorithm "back projection of filtered projections" as described previously [S8], which yielded 32-bit floating-point image-stacks with isotropic voxel size of 2.47 µm in each dimension. The image stacks were treated for optimal visualization using the open-source software FIJI [S9] and volume rendered using the open-source software Drishti v2.6 [S10]. Supplementary videos were generated with Drishti v2.6 and edited in Adobe Premiere CS6 (Adobe, San Jose, CA, USA). For scanning electron microscopy (=SEM), specimens of *Eoperipatus* sp. were fixed in 4% formalin, dehydrated in an ethanol series, dried in a critical point dryer, coated with gold in a sputter coater and examined in a scanning electron microscope (EVO 50, Carl Zeiss, Jena, Germany), as described previously [S6, S11]. Final panels and diagrams were designed with Adobe Illustrator CS6 and exported in the Tagged Image File Format (TIFF). Terminology of morphological structures is provided following Oliveira *et al.* [S6, S11, S12].

Expanded Systematic Paleontology

Onychophora Guilding, 1853 [S13]
Peripatidae Evans, 1901 [S14]
†*Cretoperipatus burmiticus* Engel & Grimaldi, 2002 [S15]

Color pattern. Pigmentation preserved in BU-001468 but not in BU-001467 or ZZZ0066 (Figure 1); ground color brown in BU-001468; no evident dorsal color pattern; ventral body surface brighter than dorsal.

Head. Paired frontal antennae followed posteriorly by well-developed, dorso-lateral eyes and lateral slime papillae; modified head papillae and/or head organ absent; proximal antennal rings covered solely with type I sensilla [S6] (Figure 2A, B and Movie S1). Antennal sensory field composed of spindle-shaped sensilla [S6] and frontal organ evident at the ventral antennal basis (Figure 3A); distal antennal portion not preserved. Ventral mouth not preserved in BU-001467 or ZZZ0066 and partially covered by onychophoran slime in BU-001468; six oral lips exposed in BU-001468 (Figure 3 and Movie S1). Single preserved jaw fragment (inner jaw blade) found separately from BU-001467 in the same amber piece and composed of a principal tooth and three accessory teeth connected via a diastema to a denticle blade composed of 11 denticles (Figures S1D and S3A); diastema bearing a diastemal membrane.

Legs. BU-001467 and ZZZ0066 showing 22 leg pairs; 12 anterior-most leg pairs preserved in BU-001468 (Movie S2); all legs bearing a distal foot equipped with paired claws and two distal foot papillae — one anterior and one posterior; claws preserved in their anatomical position in BU-001468 and ZZZ0066, but dispersed in the amber piece containing BU-001467; basal foot- and bean-shaped papillae absent (Figures 2C, 3B and S3B). Four complete ventral spinous pads per leg; fifth fragmented pad present in the legs of the midbody; spinous pads reducing in number posteriorly as following: vestigial fifth pad absent in both the penultimate and the anteriorly adjacent leg pairs, which show a fragmentation of the fourth pad instead, whereas the last leg pair lacks the fourth pad completely, showing only three pads (Figures 3C and S2A, B). Nephridial tubercle roundish and present distally in the 4th and 5th leg pairs (Figure 3D).

Integument. Dermal papillae of the dorsal integument arranged into twelve plicae per segment; seven complete plicae passing between adjacent leg pairs to the ventral body surface; five plicae restricted to the dorso-lateral body region and varying in length (dotted lines in Figure 2D); dorsomedian furrow evident along the entire antero-posterior body axis (Figure 2D and Movie S3). Dorsal plicae composed of one or two accessory papillae between two primary ones; primary papillae of the dorsal integument divided into basal and apical pieces separated by a clear constriction; apical piece equipped with a sensory bristle displaced posteriorly (Figure 2E); accessory papillae roundish, smaller than the primary ones and lacking apical piece and sensory bristle (Figure 2F); both types of papillae reduced in size and arranged randomly on the ventral integument.

Additional structures. Preventral and ventral organs roundish, situated along the ventral midline between corresponding legs; preventral organ smaller than and anterior to the ventral organ (Figure 3E). Type I crater-shaped papillae small, roundish and distributed in a random fashion on the plicae next to the legs (Figure 3F); type II crater-shaped papillae larger than type I, elongated and lying in the interplical space, forming a ventro-lateral and a ventral row of six papillae between adjacent legs. Genital opening located between the penultimate legs; structure evident only in BU-001467, appearing as a shrunken area on the ventral midline (dotted line in Figure S2A). Anal region not preserved in any specimen studied.

Previous interpretation of †C. burmiticus. In our study, specific morphological correspondences found between †*C. burmiticus* and *Eoperipatus* sp. suggest that the incomplete holotype of †*C. burmiticus* was probably misinterpreted as the anterior, rather than posterior, half of the animal. First, Grimaldi *et al.* [S15] described three complete spinous pads per leg in †*C. burmiticus*, but our data show that this condition only appears in the two posterior-most leg pairs, whereas the remaining legs exhibit four pads instead (Figure S1H and S2A–C). Notably, a similar pattern also occurs in various extant species of Peripatidae [S6, S11, S16]. Second, the so-called nephridial tubercles, which only occur in the 4th and 5th leg pairs of all onychophorans, have been neither illustrated (fig. 17 in Grimaldi *et al.* [S15]) nor precisely described in the original publication. Our data suggest that these structures would hardly be overlooked even using traditional light microscopy, given their large size and, contrary to the original claim [S15], their position in the third spinous pad, splitting it into two distinct parts (Figure S2C). Therefore, the holotype of †*C. burmiticus* probably represents a posterior body part and the structures originally interpreted as the mouth and base of the antenna/slime papilla [S15] may instead correspond to the genital opening and last leg, respectively.

Furthermore, our findings arguably resolve †*C. burmiticus* within Peripatidae by revealing characters regarded as unambiguous synapomorphies of this onychophoran subgroup [S6, S11, S17]. Since none of these unique features had been clearly demonstrated in the original species description [S18], the previous classification of †*C. burmiticus* within Peripatidae [S15] was most likely a tenuous speculation, albeit an accurate one, based on the current biogeographical distribution of the major onychophoran subgroups [S19]. The identification of these and other taxonomically informative characters in †*C. burmiticus* (Figure S4) allowed us to clarify the affinities of this fossil species within Peripatidae [S6, S11]. The presence of only one anterior and one posterior distal foot papilla in †*C. burmiticus* (Figure S1E, G), for example, is unique to representatives of the Southeast Asian Peripatidae, i.e., *Typhloperipatus* and *Eoperipatus* [S6, S17, S20], and suggests that these three taxa are closely related. Unfortunately, other characters shared by the Southeast Asian peripatids are associated with internal structures [S6, S21], which have not been preserved in †*C. burmiticus*. Hence, we consider the unique number and arrangement of distal foot papillae alone as enough evidence for resolving †*C. burmiticus* within this subgroup of Peripatidae.

Expanded evidence for the post-Gondwanan colonization of India by peripatids

Three main arguments speak in favor of a post-Gondwanan colonization of India by peripatids. First, an ancient presence of Peripatidae in India cannot be logically explained based on the current biogeographical patterns of Onychophora [S18, S22, S23]. Paleogeographical studies show that India was previously associated with landmasses corresponding to present-day Australia, Antarctica and South Africa. This association remained until the lower Cretaceous, when India first disconnected from South Africa (~140 mya) and subsequently from Australia and

Antarctica [S24, S25] (~120 mya). Apart from Antarctica, which for clear reasons hosts no living onychophorans, the other two areas currently harbor a diverse onychophoran fauna composed exclusively by representatives of Peripatopsidae [S17, S18, S26-28]. The two major onychophoran subgroups, Peripatidae and Peripatopsidae, most likely diverged from each other during the Upper Devonian (~374 mya), prior to the continental drift [S23], and therefore, it is reasonable to assume that, if any Gondwanan relict population of Onychophora ever persisted in India, it would be composed of peripatopsids like in Australia and South Africa.

Second, paleoenvironmental data suggest that, even if an onychophoran population persisted in India after its split from Gondwana, it likely would have gone extinct during the Cretaceous. The Cretaceous is considered to be one of the warmest periods since the Cambrian and is often regarded as a globally tropical period [S15]. Indeed, this could have held true for the northern-most landmasses, as indicated by the typically tropical rainforest fauna (including onychophorans) in Burmese amber [S15, S29, S30]. However, the climate might have been dryer around the tropics, with higher temperatures, arid landscapes and insufficient humidity for developing rainforests [S29]. Since India was positioned within the tropics during the Cretaceous, it is possible that suitable microhabitats were unavailable for Onychophora, as these organisms are highly susceptible to dehydration and strongly dependent on humid environments for survival [S16, S31, S32]. Moreover, India underwent a series of dramatic climate changes and intense volcanism in the late Cretaceous, while it drifted northwards [S33, S34]. Therefore, the hostile climate, in addition to drastic geological events, suggests that the survival chances of a Gondwanan relict population of Onychophora in India were fairly low during the Cretaceous. One could still argue that the single report of Onychophora from Mozambique [S27], which occupied similar latitude as India during the Cretaceous, speaks against the putative extinction of onychophorans in the insular India. However, onychophorans may only have arrived in Mozambique after the Cretaceous, when suitable climate and vegetation conditions enabled peripatopsids from South Africa to gradually move northwards to Mozambique.

Finally, onychophorans have never been reported from Madagascar, even though this landmass shares a similar paleogeographical history with India. Madagascar split from the remaining Gondwana together with India (~135 mya) and stayed associated with the latter for approximately 50 million years [S24], until they finally separated from each other during the Upper Cretaceous (~87 mya). In the event that a Gondwanan relict population of Onychophora, irrespective of whether it belonged to Peripatidae or Peripatopsidae, had persisted in India and endured geological and climate events of the Cretaceous, one would expect the same to have occurred in Madagascar. However, Madagascar has been extensively sampled in the last decades and not a single onychophoran has ever been reported from the island [S26, S27]. The current lack of Onychophora in Madagascar as opposed to India might be explained, however, by the past isolation of this island. While India collided with Asia during the Cenozoic, enabling peripatids from Southeast Asia to move into this region, Madagascar has remained isolated from any continental landmass since the Upper Cretaceous, thus preventing onychophorans from reaching this area.

SUPPLEMENTAL REFERENCES

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