Current Biology

Beetle Pollination of Cycads in the Mesozoic

Highlights

- A specialized beetle-mediated pollination mode is reported from Burmese amber

- The mid-Cretaceous boganiid beetle has many pollen-feeding adaptations

- The fossil boganiid was probably a pollinator of Encephalarteae cycads

- This suggests a probable ancient origin of beetle pollination of cycads in the Mesozoic

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In Brief

Cai et al. report a new mid-Cretaceous boganiid beetle with specialized pollen-feeding adaptations. The phylogenetic analyses and associated pollen grains indicate that it was probably a pollinator of early cycads. This suggests an ancient origin of beetle pollination of cycads long before the rise of flowering plants.

CellPress

Beetle Pollination of Cycads in the Mesozoic

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SUMMARY

Cycads, unlike modern wind-pollinated conifers and *Ginkgo*, are unusual in that they are an ancient group of gymnosperms pollinated by insects [1–3]. Although it is well documented that cycads were diverse and abundant during the mid-Mesozoic, little is known about their biogeography and pollination before the rise of angiosperms. Direct fossil evidence illuminating the evolutionary history of cycads is extremely rare [4, 5]. Here we report a specialized beetle-mediated pollination mode from the mid-Cretaceous of Myanmar, wherein a new boganiid beetle, *Cretoparacucujus cycadophilus*, with specialized pollen-feeding adaptations in its mouthparts and legs, was associated with many pollen grains of *Cycadopites*. Phylogenetic analyses indicate *Cretoparacucujus* as a sister group to the extant Australian *Paracucujus*, which pollinate the cycad *Macrozamia riedlei*. Our discovery, along with the current disjunct distribution of related beetle-herbivore (tribe Paracucujini) and cycad-host (tribe Encephalarteae) pairs in South Africa and Australia, indicate a probable ancient origin of beetle pollination of cycads at least in the Early Jurassic, long before angiosperm dominance and the radiation of flowering-plant pollinators later in the Cretaceous.

RESULTS AND DISCUSSION

Cycads (Cycadales) are a non-speciose group of ancient living seed plants with remote roots in the Permian [1]. They are remarkable gymnosperm remnants, dominating terrestrial ecosystems during the Mesozoic and dwindling to their current diversity of approximately 330 species as angiosperms rose to dominance [2]. Cycads are dioecious gymnosperms, and most, if not all, share obligate mutualisms with specialist insect pollinators such as beetles (Coleoptera) and thrips (Thysanoptera) [3]. Understanding pollination modes of Mesozoic cycads is significant for elucidating the early diversification of cycads and insect-plant associations before angiosperm dominance.

With >380,000 named living species constituting almost 25% of all known lifeforms on our planet, beetles exhibit an astonishing morphological, taxonomic, and ecological diversity [6]. The beetle family Boganiidae is a small, monophyletic, and relict cucujoid group, with six extant genera and 15 species restricted to southern Africa, southwestern and eastern Australia, and New Caledonia [7]. Adult boganiids, characterized by head frons with median endocarina and mandibles with a dorsal setose cavity near the base, are distinctive among Cucujoidea [7]. Boganiidae currently comprise two subfamilies: Boganiinae (two genera) and Paracucujinae (four genera). In Paracucujinae, two closely related but geographically widely separated genera, *Metacucujus* and *Paracucujus* (tribe Paracucujini), feed on cones of the cycad tribe Encephalarteae (Zamiaceae) in southern Africa and the southwest of Western Australia [4, 7, 8]. This distribution suggests a Gondwanan vicariance during the Middle Jurassic resulting in the isolated distribution for these poorly dispersing beetles and their cycads [4, 9]. Here, we report on a cucujoid beetle belonging to the Boganiidae preserved within a piece of mid-Cretaceous Burmese amber (approximately 99 Ma [10]), which also harbors many cycad pollen grains alongside the beetle. The phylogenetic placement of the beetle along with the presence of specialized mandibular cavities known in modern cycad-pollinating boganiids for the transport of pollen reveal the fossil to be an early-cycad-visiting species. This specialized beetle-gymnosperm association represents the first probable insect pollination mode for Cycadales during the mid-Mesozoic.

Systematic Paleontology
Order Coleoptera Linnaeus, 1758.

Family Boganiidae Sen Gupta and Crowson, 1966.

Subfamily Paracucujinae Endrödy-Younga and Crowson, 1986.

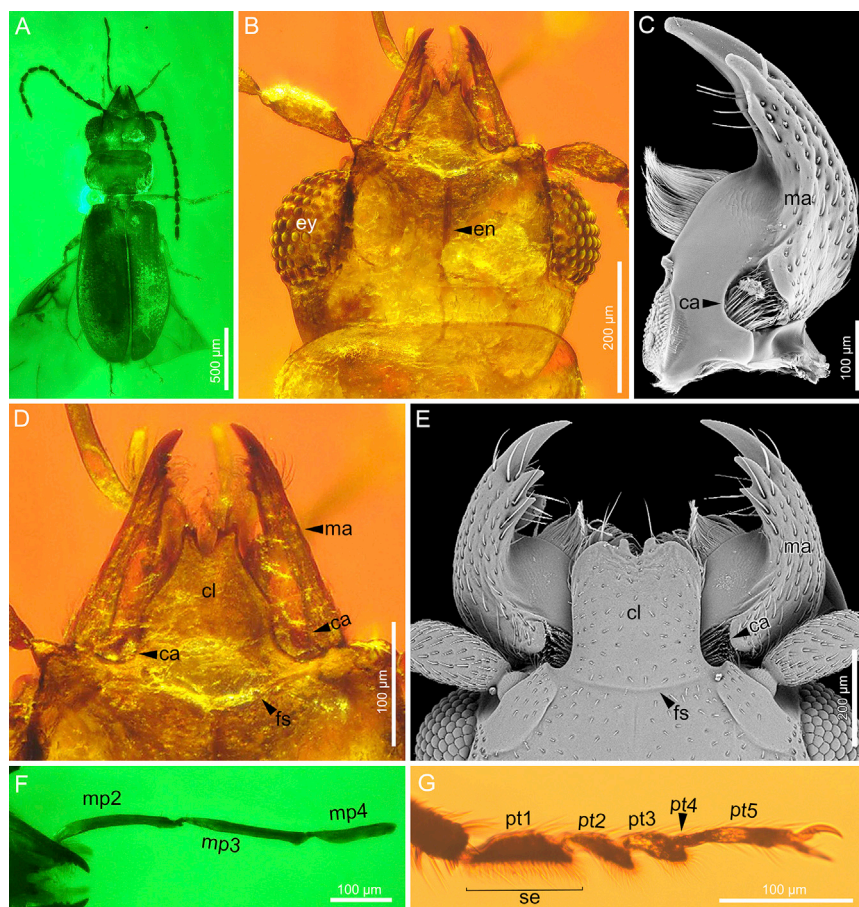*Cretoparacucujus cycadophilus* gen. et sp. nov. Cai and Escalona.

Figure 1. Photomicrographs of *Cretopara-cucujus cycadophilus* and Mouthparts of Extant *Paracucujus rostratus*

(A) General habitus, dorsal view, under epifluorescence.

(B) Head of *C. cycadophilus*, dorsal view, under normal reflected light.

(C) Right mandible of *P. rostratus*, showing the characteristic setose mandibular cavity, under scanning electron microscope (SEM).

(D) Mouthparts of *C. cycadophilus*, dorsal view, under normal reflected light.

(E) Mouthparts of *P. rostratus*, dorsal view, under SEM.

(F) Left maxillary palpus of *C. cycadophilus*, under epifluorescence.

(G) Right protarsus of *C. cycadophilus*, lateral view, under reflected light.

Abbreviations: ca, mandibular cavity; cl, clypeus; en, endocarina; ey, compound eye; fs, frontoclypeal sulcus; ma, mandible; mp2–4, maxillary palpomeres 2–4; pt1–5, protarsomeres 1–5; se, adhesive setae. See also Figures S1 and S2.

Material

Holotype, NIGP166883, female. Mid-Cretaceous amber (ca. 99 million years ago), Tanai, Kachin State, northern Myanmar.

Etymology

The generic name is a combination of *creto-* and the genus *Paracucujus*. The specific epithet is a combination of Greek *kykas* (meaning, cycad) and *philia* (meaning, friendly love or affection).

Diagnosis

Cretoparacucujus is distinguished from other boganiids by the following combination of characters: upper body surface subglabrous; head large, slightly wider than pronotum; antenna filiform, without antennal club; clypeus sub-triangular, apex widely notched medially; frontal carina meeting frontoclypeal sulcus; mandible long, nearly straight; maxillary palpus elongate, with maxillary palpomere 4 much shorter than palpomere 3; protibial apex not expanded; and elytral punctation seriate.

Description

Refer to Data S1 for a complete description.

The fossil species *Cretoparacucujus cycadophilus* (Figures 1A, 2, S1A, and S1B) displays many diagnostic morphological features of Boganiidae, including mandibles with large, setose cavities on the dorsal surface (synapomorphy of the family; Figures 1B and 1D), elongate clypeus (Figure 1D), and tarsi with a greatly reduced tarsomere 4 and ventrally lobed tarsomere 3 (Figures 1G and S2F) [7]. *Cretoparacucujus cycadophilus* can be further placed in one of the two extant subfamilies, namely Paracucujinae, by the lateral pronotal carina without an anterior callosity, long and parallel-sided apical maxillary palpomere (Figure 1F), and seriate elytral punctation (Figures 1A and S1A). To investigate the precise phylogenetic position of the new genus, we analyzed an integrated morphological data matrix combing our fossil and representatives of all extant genera using both parsimony and Bayesian approaches. Results under parsimony, using both equal weights and implied weighting, all coincided in the monophyly of Boganiidae. All analyses strongly supported *Cretoparacucujus* as a sister group to *Paracucujus*, and they together as sister to *Metacucujus* (Figure 3). The alternative Bayesian analysis produced a similar tree, supporting *Cretoparacucujus* as a sister group to *Paracucujus* (Figure 3). To date, Paracucujinae comprise four genera (*Athertonium*, *Dzumacium*, *Metacucujus*, and *Paracucujus*) [7]; *Cretoparacucujus* can be assigned to the derived *Metacucujus* + *Paracucujus* clade based on a single synapomorphy, i.e., filiform antennal flagellum without distinct antennal club (Figure S1C) [7]. A close affinity with the Australian *Paracucujus* is supported by the striate elytra and apex of clypeus with small teeth [7].

The most impressive feature of *Cretoparacucujus* is the large prognathous head with large compound eyes, sharp mandibles, and extremely long maxillary palpi (Figures 1A, 1B, 1F, S1D, S1F, and S2A). These features, close to those of
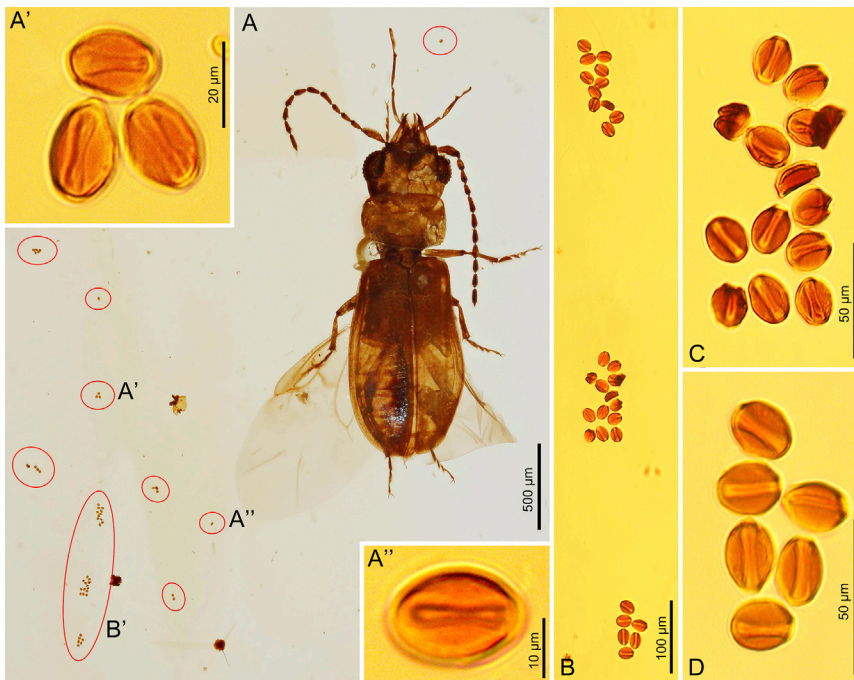
Figure 2. Photomicrographs of Cycad Pollen Grains Associated with *C. cycadophilus*

(A) General view of *C. cycadophilus* and aggregations of pollen grains by the beetle.

(A') Enlargement of an aggregation of three pollen grains.

(A'') Enlargement of a single grain.

(B) Enlargement of three larger aggregations of pollen grains.

(C) Enlargement of (B), showing 14 aggregated pollen grains.

(D) Enlargement of (B), showing six aggregated pollen grains.

Paracucujus (Figures 1E, S1E, and S1G) and the Jurassic *Parandrexis* (Parandrexidae), probably suggest an open habitat, and most likely habitation on the surface of the strobiles of gymnosperms [11]. The mandible of *Cretoparacucujus* bears a dorsal, round, and setose cavity (Figures 1D and S1D) near its base, and the clypeus is basally constricted (Figure 1D), making the mandibular cavity clearly visible in dorsal view. This specialized modification of the mandible (Figure 1C) has been known to function in containing and most likely transporting pollen grains, as both adults and larvae of extant Boganiidae feed on pollen [7, 12]. In Cucujoidea, similar analogous mandibular cavities are known in the extant pollen beetles (Nitidulidae: Meligethinae), which are pollenophagous and possible pollinators [13]. Observations of the mandibles of some extant boganiids such as *Athertonium* and *Boganium* have provided direct evidence that the peculiar mandibular cavities are capable of carrying a few pollen grains [13]. As in most boganiids, the tarsi of *Cretoparacucujus* have dense normal adhesive setae on the ventral surfaces of the basal three tarsomeres (Figures 1G and S2F), which is a specialization for holding and climbing on the surfaces of plants. In addition, the maxilla of *Cretoparacucujus*, with an apically expanded and densely setose galea (Figure S2D), is typical of many modern boganiids, and it may be used to collect small particles such as pollen and direct them into the mouth.

In Paracucujinae, the life history of *Dzumacium* (New Caledonia) is unknown, and their feeding habit remains elusive. Adults of *Athertonium* from eastern Australia are pollenophagous, associated with angiosperms such as Lauraceae, Elaeocarpaceae, Cunoniaceae, and Meliaceae [7]. Species of *Metacucujus* from South Africa are dependent on male cones of various cycads in the genus *Encephalartos* [7]. There is evidence indicating that *Metacucujus encephalarti*, as well as an erotylid beetle, are probably the main pollinators of *Encephalartos* [8, 14]. The

monotypic genus *Paracucujus* from southwestern Australia occurs in large numbers on male cones of *Macrozamia riedlei* and sometimes in sticky traps on female cones [7, 13]. As such, *Paracucujus rostratus* is probably a pollinator of *Macrozamia riedlei* [3, 13]. Recent phylogenetic analyses indicated that the extant *Metacucujus* and *Paracucujus* are close extant sister groups [7], as are the two associated cycad host-plant genera, which belong to the same tribe, Encephalarteae (Zamiaceae). This remarkable distribution indicates a Gondwanan vicariance that began during the Middle Jurassic for these poorly dispersing beetle and cycad pairs [4, 9]. Apparently, the beetle-cycad interaction was established during the Mesozoic as supported by the disjunct distribution of the beetle and cycad pairs. Our discovery of a *Paracucujus*-related genus from the Cretaceous suggests that *Cretoparacucujus* may have a similar feeding habit to that of *Paracucujus*, feeding on pollen grains of cycads, as this seems to be a biological trait for the clade as a whole.

It is remarkable that several aggregations of exquisitely preserved pollen grains are located along the left side of the fossil beetle and with two pollen grains close to the head (Figure 2). All pollen grains associated with *C. cycadophilus* are boat shaped, prolate, and monosulcate. They display an elliptical outline and rounded polar margin in polar view and subcircular shape in equatorial view. Average polar axis length and equatorial diameter of the palynospecies (N = 21) are 20.65 μm (20.07–21.04 μm) and 14.30 μm (14.20–14.37 μm), respectively. The length-width ratios range from 1.1 to 1.4. The sulcus is elongate, extending almost the entire length of the grain. The sulcus exhibits rounded ends and it is much broader at their ends (~4.62 μm) than at the mid-point (~2.12 μm). The ornamentation is psilate, and the pollen exine is approximately 1 μm thick. Based on a combination of the shape, sulcus structure, and ornamentation, the pollen can be referred to *Cycadopites*, a form-genus of polyphyletic origin occurring in sediments from the late Palaeozoic to Holocene [1], which can be also produced by modern cycads. It is challenging to affiliate *Cycadopites* that are taphonomically deformed to a particular group of gymnosperms, as they are comparable to pollen grains of a wide range of plants, including Bennettitales, Cycadales, Czekanowskiales, Ginkgoales, Peltaspermales, Pentoxylales, and a few basal angiosperm lineages. However, these amber-entombed pollen
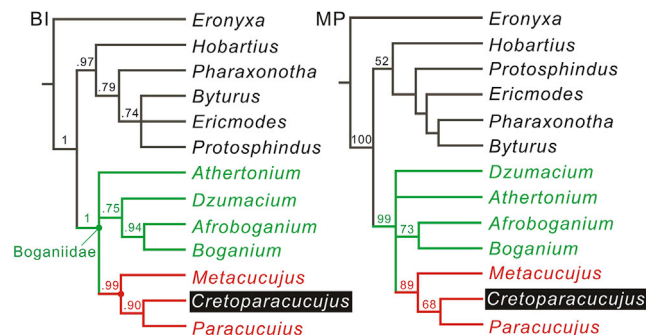
Figure 3. Phylogenetic Positions of *Cretoparacucujus* from Bayesian Inference and Maximum Parsimony Analyses

Posterior probabilities above 0.5 are shown on branches in the Bayesian inference (BI) tree (left); bootstrap support values over 50% are shown on the maximum parsimony (MP) tree (right) (strict consensus of two trees: length = 121 steps, consistency index [CI] = 0.65, retention index [RI] = 0.67). See also Figure S3, Table S1, and Data S1.

grains exhibit three-dimensional features with high fidelity that allow for a more accurate systematic attribution. These pollen grains differ from those of Ginkgoales by their oval outline, rounded ends, and the colpus form [15]. Some pollen grains of extinct Bennettitales also belong to *Cycadopites*, but they are distinguished by their large size and a more spindle-like outline [15]. The present pollen grains are comparatively small in size (~21 μm long), close to some species of *Cycas*, *Macrozamia*, and *Zamia*, but considerably smaller than those of *Encephalartos* and *Lepidozamia* [16].

Another fact supporting this beetle-mediated pollination is that these *Cycadopites* pollen grains are in multiple aggregations, which comprise 3–14 grains in the specimen (Figures 2A' and 2B–2D). Many modern entomophilous cycad pollens and pollens of insect-pollinated angiosperms adhere in large aggregations, whereas wind-pollinated, or anemophilous, pollens are dispersed as single grains, or monads [17, 18]. The Burmese amber has yielded a diverse Cretaceous flora including moss, liverworts, ferns, conifers, and angiosperms, but cycads remain unknown. Our discovery of the *Cycadopites* pollen represents the first evidence of cycads from Burmese amber.

Given the feeding functional morphology and phylogenetic placement of *Cretoparacucujus*, as well as the identification of the associated cycad pollen aggregations, the Cretaceous *C. cycadophilus*, like the related *P. rostratus*, was probably a cycad pollen feeder and most likely a pollinator for *Macrozamia*-related cycads that belong to the tribe Encephalarteae (Figure S3). Moreover, the establishment of this type of boganiid-cycad association may be significantly older, probably extending back prior to the eventual breakup of Gondwana during the Early Jurassic, some 167 million years ago [19]. The hypothesis is also supported by the remarkable vicariance of two poorly dispersing cycad-host and beetle-herbivore pairs from widely separated Gondwanan continents (Figure 4) [9]. The species of *Encephalartos* from southern Africa and *Macrozamia riedlei* from Western Australia, belonging to the same tribe Encephalarteae (Zamiaceae), are closely related [20], so are their corresponding pollenophagous beetles: *Metacucujus* and its modern sister group, *Paracucujus* [7].

Cycads have a rich fossil record, extending to the earliest Permian [21] and peaking in diversity during the Jurassic and Cretaceous periods [1]. Unfortunately, fossil cycads are usually preserved as fragmentary stems or leaves, and rarely with reproductive organs, which yield non-overlapping character datasets, hindering phylogenetic analyses [22]. The earliest cycad with an affinity to Zamiaceae, *Plagiozamites oblongifolius*, comes from Late Permian of China [23]. Its stomatal architecture, especially the prominent thickenings of the subsidiary cells, resembles that of some extant *Macrozamia* and *Encephalartos* [23], both belonging to the entomophilous Encephalarteae. More convincing fossils placed in Encephalarteae are from the Cretaceous of Antarctica, India, and Patagonia [22]. Cenozoic representatives of Encephalarteae from the early Eocene of Patagonia and the middle Eocene of Australia suggest that the tribe occurred widely across Gondwanan landmasses until the final breakup of the former supercontinent [22].

Recent fossil-calibrated molecular dating estimates produced various ages for the origin of crown cycads, ranging from ca. 200 Ma (Early Jurassic) to 274.5 Ma (Late Permian), but all suggested that the extant diversity of cycads derives from mostly Miocene radiations and postdates the Cretaceous-Paleogene boundary [2, 20, 24]. Encephalarteae, as traditionally defined based on morphology, was recovered as a monophyletic group by almost all recent molecular phylogenies [2, 20, 24], except in a maximum-likelihood analysis based on a single nuclear gene [2]. It is unexpected that these molecular divergence estimates suggested an origin of stem-group Encephalarteae during the Late or Early Cretaceous [2, 20, 24]. This young age for origin appears to be unlikely, because at that time Africa (western Gondwana) was widely separated from eastern Gondwana, which comprises Antarctica, India, Madagascar, Australia, and New Zealand [19]. Considering the low dispersal ability of early cycads and their potential beetle pollinators, Encephalarteae and their cycad-beetle associations were most likely established before the separation of the Gondwanan landmasses, a time during the Early Jurassic or earlier. Additionally, fossil evidence of late Permian cycad cuticles and a late Triassic whole-plant from China [23, 25], both with apomorphies of modern Encephalarteae, suggest a much longer evolutionary history for the tribe than implied by methods relying solely on indirect inferences from molecular clocks.

Modern cycads are principally pollinated by beetles, and rarely by thrips or moths [3]. Their beetle pollinators mainly include cucujoids (Biphyllidae, Boganiidae, Erotylidae, and Nitidulidae), weevils (Anthribidae, Belidae, Brentidae, and Curculionidae), and, unusually, tenebrionids [3]. *Cycadothrips*, a lineage of basal thrips, participate in the pollination of some species of *Macrozamia* in Australia. Based on the biogeography of *Cycadothrips* and the antiquity of the family to which it belongs (Aeolothripidae), thrips were most likely among the earliest pollinators of these plants [4, 26], although fossil evidence is lacking. Weevil pollinators of cycads, as revealed by extensive comparative studies, are probably derived from angiosperm-dwelling ancestors, rather than from the older gymnosperm feeding weevils [27], and are therefore correspondingly and comparatively younger in age. It is noteworthy that the Triassic obrieniids with a weevil-like rostrum (nose) and their frequent co-occurrence with cycad remains have been hypothesized as evidence of a potential Triassic beetle-cycad association [28]. Such early interactions have been
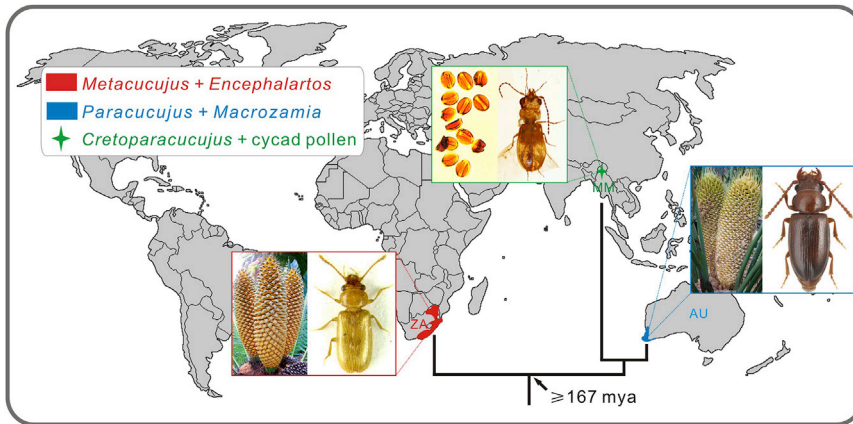
Figure 4. Geographic Distribution of the Known Entomophilous Cycads of the Tribe Encephalarteae and Their Boganiid Pollinators

The phylogenetic relationships and divergence time of the widely separated lineages within Boganiinae are shown. The arrow indicates the divergence time estimated by separation of Gondwana in the Jurassic. Abbreviations: AU, Australia; MM, Myanmar; ZA, South Africa. See also Figure S3 and Table S1.

exemplified by a Middle Triassic cycad cone that contains pollen-laden coprolites possibly produced by beetles [28]. This find provides the earliest evidence for a cycad-insect interaction, illuminating the early stage in the establishment of complex entomophily in cycads [28]. Among the cucujoid pollinators, Nitidulidae have their oldest known fossil records from the Early Cretaceous, whereas Biphyllidae and Erotylidae date back to the mid-Eocene [29–31]. By contrast, the first boganiid fossil, *Palaeoboganium jurassicum*, has been recently documented from the Middle Jurassic (approximately 165 Ma) of northeastern China [32]. Morphology-based phylogenetic analyses recovered *Palaeoboganium* as a sister group to *Metacucujus + Paracucujus*, which implied the fossil beetle as "a good candidate" for a pollinator of cycads [32]. The feeding habits of *P. jurassicum* are undoubtedly difficult to determine due to the insufficient preservation of compression fossils (e.g., lacking details of mandible and gut contents), and its position as basal to the two genera pollinating cycads leaves its biology somewhat ambiguous. Nevertheless, *P. jurassicum* demonstrates that Boganiidae were present during that period, originating at least as early as the Middle Jurassic, and were more widespread than initially believed. Biogeographically, Boganiidae may have also occurred across Gondwana during the Middle-Late Jurassic, as similar distribution patterns have been observed in multiple beetle lineages as shown by fossils from the Jurassic of China and Australia [33]. The Boganiidae-Encephalarteae association was probably established during the Early-Middle Jurassic, and the specific beetle-herbivore and cycad-host pairs were probably widespread on Gondwana, at least in what would become southern Africa, southwestern Australia, and Antarctica, the last then connecting the former two continents [34]. Like boganiids, the present global distribution of pollinating erotylids in Africa, Asia, Australia, and North and Central America [3] suggests an early development of an erotylid-cycad relationship before late-Mesozoic continental drift [35]. Collectively, the preliminary cycad-insect associations are probably ancient, extending to the Triassic or earlier [28], whereas insect pollination of cycads may be as early as the Early Jurassic [4, 9, 12], long before the origin and diversification of major groups of angiosperms and the concomitant diversification of angiosperm pollinators such as moths, flies, and bees later in the Cretaceous [5, 6, 36, 37]. Moreover, such an ancient association was probably widespread at least

across Gondwana in the Mesozoic, as evidenced by Cretaceous cycads of the Encephalarteae from Antarctica, India, and South America [22].

Our discovery of a mid-Mesozoic insect pollinator of cycads provides a window into ancient insect-gymnosperm interactions alongside the diversification of angiosperms. Unlike some beetles that shifted plant-hosts from gymnosperms onto angiosperms during the Aptian-Albian gap [5], the beetle pollination of Zamiaceae cycads has most likely persisted from the early Mesozoic to the present, although confined to particular areas in the Southern Hemisphere. Their austral disjunct occurrence resulting from vicariance during the Jurassic followed by considerable stasis in pollination biology over the intervening epochs all in the face of dramatic global climate change, shifting landmasses, and considerable declining diversity among cycads and other major gymnosperm lineages.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Specimen imaging
  - Phylogenetic analysis
- DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, one table, and one data file and can be found with this article online at https://doi.org/10.1016/j.cub.2018.06.036.

AUTHOR CONTRIBUTIONS

C.C. conceived and designed the study. C.C. drafted the manuscript, to which M.S.E., Z.Y., and H.E.E. contributed. C.C., Z.Y., H.E.E., D.H., and M.S.E participated in the morphological studies of the boganiid fossil. L.L. and C.C. participated in the studies of the cycad pollen grains. C.C. conducted the phylogenetic analyses.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
Helicon Focus 3.10	Helicon Soft	https://www.heliconsoft.com/
MrBayes 3.2.3	[38]	http://mrbayes.sourceforge.net
Tracer version 1.6.0	Tracer Website	http://tree.bio.ed.ac.uk/software/tracer/
TNT 1.5	[39]	https://cladistics.org/tnt/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Chenyang Cai (cycai@nigpas.ac.cn).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The Burmese amber specimens studied here are derived from the Hukawng Valley in Tanai Township, Myitkyina District of Kachin State, Myanmar. The holotype (NIGP166883) is deposited in the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing, China. The new taxonomic actions established herein are registered in ZooBank LSID urn:lsid:zoobank.org:pub: A05BB478-ECFE-4797-8FC2-86536C84A8E3. The amber piece was polished with sand papers of different grits and diatomite mud.

METHOD DETAILS

Specimen imaging
Photomicrographs were taken using a Zeiss Discovery V20 microscope system, and those with green background (Figures 1A and 1F) used fluorescence as a light source attached to a Zeiss Axio Imager 2 compound microscope. Focus stacking software (Helicon Focus 3.10) was used to increase the depth of field.

Phylogenetic analysis
We scored *Cretoparacucujus cycadophilus* sp.nov. for 63 adult characters and integrated the fossil taxon along with representatives of extant and extinct genera of Boganiidae, their related cucujoid genera, and a cleroid outgroup. Most characters (60 out of 63) of extant genera of Boganiidae, their related cucujoid genera, and an outgroup were derived from Escalona et al. [7] We added three new characters to the matrix that are relevant to the diagnosis of *Cretoparacucujus* (first three characters in the character list in Supplementary Information). We excluded the larval characters used by Escalona et al. [7], as larvae of the fossil taxa remain unknown. The character states and matrix are presented in Table S1. All characters were non-additive, unordered, and equally weighted. Bayesian analysis was carried out using MrBayes 3.2.3 [38] and the discrete Mkv+G model [40]. Two MCMC runs of four chains were run for three million generations. The consensus tree was estimated after a burn-in of 25% of the sampled trees. Convergence was confirmed with Tracer version 1.6.0. Parsimony analysis was conducted using TNT 1.5 [39] using New Technology search. We used two search strategies, including equal weights and implied weights. For the implied weights analyses, we tested a set of concavity (k) values from 0.25 to 10 and found no changes to the tree topology as it relates to Boganiinae. Branch support values were estimated using 10,000 bootstrap replicates.

DATA AND SOFTWARE AVAILABILITY

The detailed systematic paleontology for *Cretoparacucujus cycadophilus*, taxon selection and morphological characters used for phylogenetic analyses are available in Data S1.

Supplemental Information

Beetle Pollination of Cycads in the Mesozoic

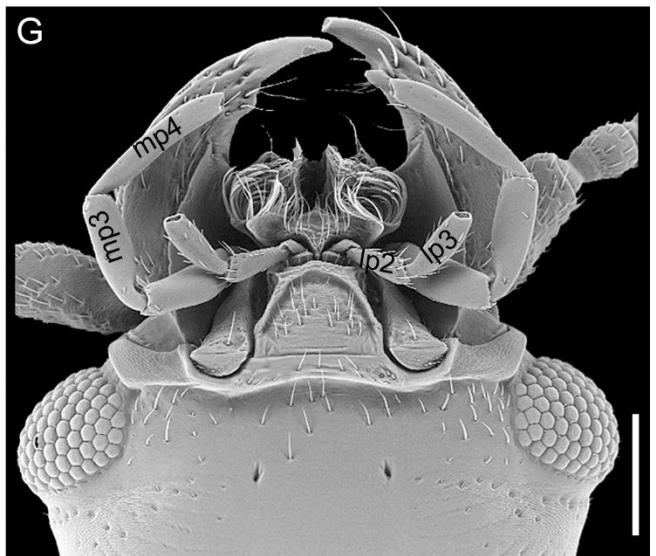**Chenyang Cai, Hermes E. Escalona, Liqin Li, Ziwei Yin, Diying Huang, and Michael S. Engel**
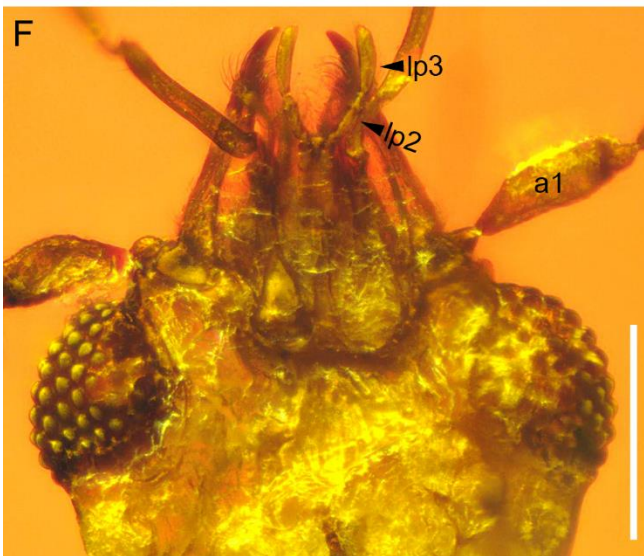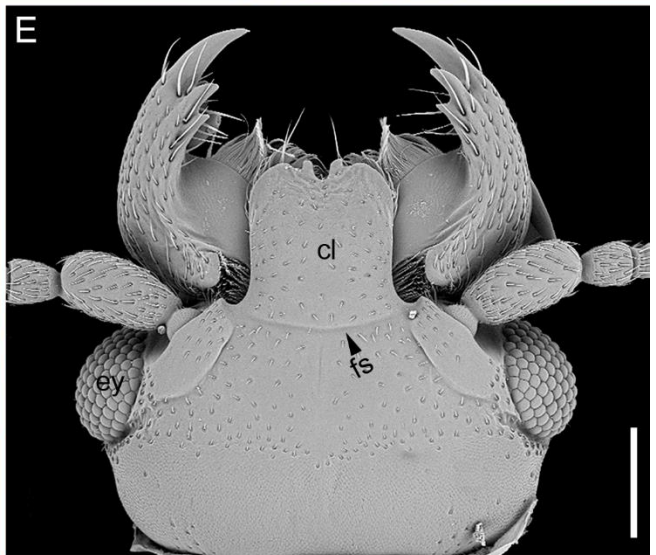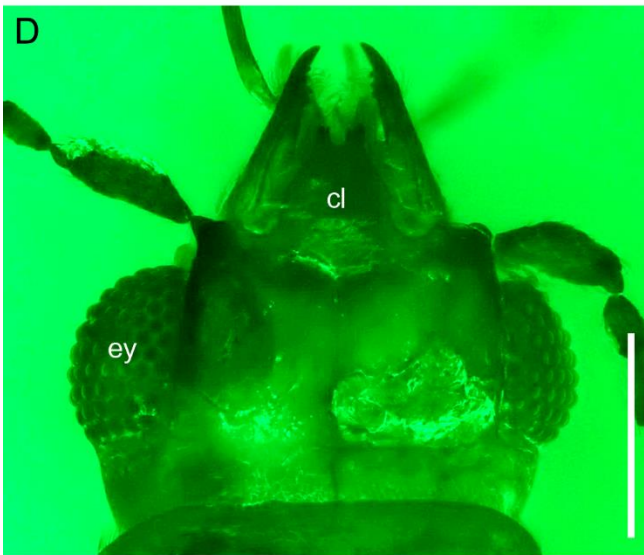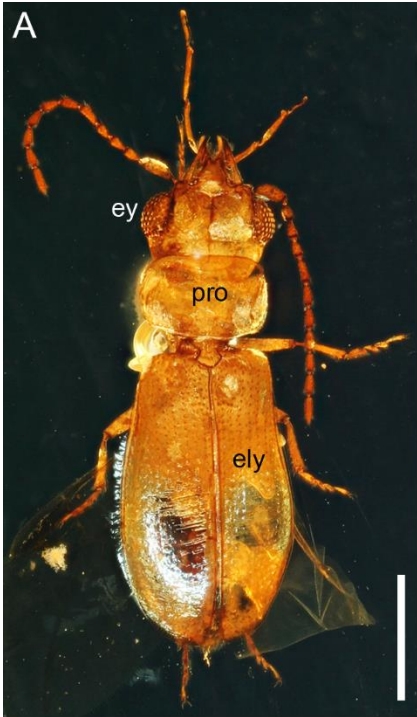
Figure S1. Photomicrographs of *Cretoparacucujus cycadophilus* (NIGP166883) and Mouthparts of Extant *Paracucujus rostratus*. Related to Figure 1

(A) General habitus, dorsal view, against black background.

(B) General habitus, ventral view, against white background.

(C) Left antenna, under reflected light.

(D) Detail of head, dorsal view of *C. cycadophilus*, under epifluorescence.

(E) Detail of head, dorsal view of *P. rostratus*, under scanning electron microscope (SEM).

(F) Detail of head, ventral view of *C. cycadophilus*, under reflected light.

(G) Detail of head, ventral view of *P. rostratus*, under SEM.

Abbreviations: a1, antennomere 1; cl, clypeus el, elytron; ey, compound eye; fs, frontoclypeal sulcus; hw, hind wing; lp2,3, labial palpomeres 2,3; mp, maxillary palpus; mp3,4, maxillary palpomeres 3,4; pro, pronotum. Scale bars: 500 µm in (A) and (B); 200 µm in (C)–(G).

A

mp4

mp3

mp2

B

mp4

mp3

mp2

D

C

hw

el

E

F

mst1

mst2 mst3 mst4 mst5

G
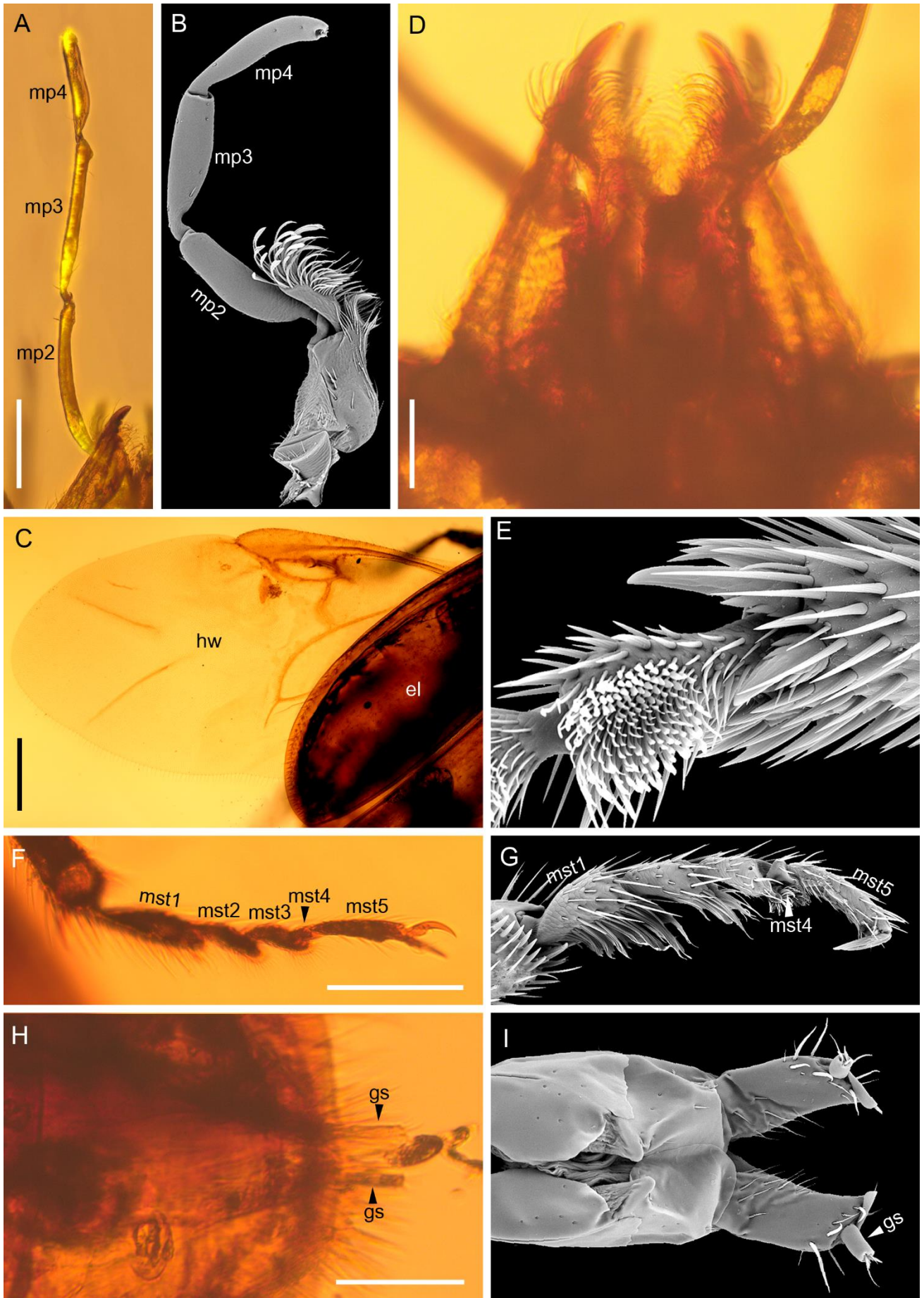
mst1

mst5

mst4

H

gs

gs

I

gs

Figure S2. Details of *C. cycadophilus* (under normal transmitted light) and *P. rostratus* (Under SEM). Related to Figure 1

(A) Left maxillary palpus of *C. cycadophilus*.

(B) Left maxillary palpus of *P. rostratus* for comparison.

(C) Left hind wing of *C. cycadophilus*.

(D) Maxillae of *C. cycadophilus*, ventral view.

(E) Adhesive setae on the first mesotaromere of *P. rostratus*.

(F) Right mesotarsus of *C. cycadophilus*, lateral view.

(G) Mesotarsus of *P. rostratus*, lateral view.

(H) Genitalia of *C. cycadophilus*, female.

(I) Female genitalia of *P. rostratus*.

Abbreviations: el, elytron; gs, gonostylus; hw, hind wing; mp2–4, maxillary palpomeres 2–4; mst1–5, mesotarsomeres 1–5. Scale bars: 100 µm in (A), (D) and (F); 200 µm in (C); 50 µm in (H).

Figure S3. Ecological Reconstruction of the Mid-Cretaceous *Cretoparacucujus cycadophilus*.

Related to Figure 4

Characters \ Taxa	1 0	2 0	3 0	4 0	5 0	6 0
Eronyxa	112112221321211233221211112212121211212111211321111113211312231
Byturus	112112111321311112221311112213212312111111321121212123121312122
Ericmodes	112112331321211113221111212214121311111112321221212113121212112
Protosphindus	111112331321132113221211112314121311121121322132122111212111211 2112
Pharaxonotha	111112111321211113222111232222221222111222421211212112211112211
Hobartius	112111231321212112222111212111213121121221232122112111311211111
Metacucujus	112122122211332231111221112212111212112212321221113121212312112
Paracucujus	211122122211332231111221112212121212112212321221113121212312112
Athertonium	111112122312332231111232112212121212112212321221113322222312112
Dzumacium	112232122311332231111231112212121212112122123212 2?11312?222312122
Boganium	112232122311332231221233112112121212112212321221113222222312122
Afroboganium	112132122311132231221233112112121212112212321221 22?112222212312122
Cretoparacucujus	221222122111332?31111?21112212?21212112212321?211131????????1?2

Table S1. Character matrix for phylogenetic analyses, including 63 adult morphological characters. Related to Figure 3

Data S1

1. Systematic Paleontology

Order: Coleoptera Linnaeus, 1758
Family: Boganiidae Sen Gupta and Crowson, 1966
Subfamily: Paracucujinae Endrödy-Younga and Crowson, 1986

Cretoparacucujus **gen. nov. Cai and Escalona**
ZooBank LSID: urn:lsid:zoobank.org:act:578961B8-5BDC-4FFF-92FC-8F0F6FB65B58.

Type species. *Cretoparacucujus cycadophilus* sp. nov.

Etymology. The generic name is a combination of *cretos* (meaning, "chalk," and from which the period name, Cretaceous, is based), and the related genus *Paracucujus*; the gender of the name is masculine.

Diagnosis. *Cretoparacucujus* is distinguished from other boganiids by the following combination of characters: upper body surface sub-glabrous; head large, slightly wider than pronotum; antenna filiform, without antennal club; clypeus sub-triangular, apex widely notched medially; frontal carina meeting frontoclypeal sulcus; mandible long, nearly straight; maxillary palpus elongate, with maxillary palpomere 4 much shorter than palpomere 3; protibial apex not expanded; and elytral punctation seriate.

Description. Female. Body small (ca. 2.0 mm long), elongate, about 3.0 times as long as wide (Figure 1A and Figure S1A, B).

Head slightly constricted posteriorly, widest across compound eyes (Figure 1B and Figure S1D). Vertex with transverse line (Figure 1B and Figure S1D). Frontal region with median endocarina extending from vertex to frontoclypeal sulcus (Figure 1B and Figure S1D). Compound eyes large, entire, strongly protuberant, coarsely facetted, interfacetal setae not evident (Figure 1B and Figure S1D, F). Antennal insertions clearly concealed by frontal ridges (Figure S1D); subantennal grooves absent. Frontoclypeal sulcus present, straight, weakly curved (Figure 1B and Figure S1D); apex of clypeus widely notched, each side bidentate (Figure 1D). Labrum concealed in dorsal view. Antenna with 11 antennomeres, filiform (Figure S1C). Mandible elongate, nearly straight; apex unidentate, with three or more small subapical teeth, dorsally with basal setose cavity (Figure 1D). Maxilla with elongate, setose galea and short lacinia (Figure S2D); maxillary palpus elongate, maxillary palpomere 4 distinctly shorter than maxillary palpomere 3 (Figure 1F and Figure S2A); labial palpus short, labial palpomere 3 fusiform, distinctly longer and wider than labial palpomere 2.

Pronotum strongly transverse, widest at middle, slightly narrower than head; sides slightly curved, not explanate; lateral pronotal carinae complete, visible for their entire lengths from above; posterior angles obtuse; posterior edge finely margined; disc without sublateral carinae. Prosternum in front of coxae slightly longer than shortest diameter of coxal cavity. Prosternal process not visible. Procoxa not projecting below prosternum; protrochantin exposed. Procoxal cavities transverse, apparently separated, apparently open. Mesoscutellar shield not elevated, laterally expanded, posteriorly broadly rounded.

Elytra complete, with about 10 puncture rows plus scutellary striole (Figure S1A). Elytral epipleura incomplete. Mesoventrite short, with paired procoxal rests. Mesocoxa not projecting. Mesocoxal cavities narrowly separated, open laterally. Metaventrite with discrimen elongate (Figure S1B); postcoxal lines absent; exposed portion of metanepisternum long and narrow. Metacoxae narrowly separated, not extending laterally to meet elytra; metacoxal plates absent. Hind wing with

long apical field containing small dark, post-radial sclerite just beyond radial cell and two radial vein remnants almost reaching wing margin; radial cell elongate with inner posterobasal angle acute; r3 short; basal portion of RP moderately long; wedge cell absent (Figure S2C).

Legs well developed; trochanterofemoral joint oblique with base of femur separated from coxa; tibial apices slightly widened; tarsal formula 5-5-5; tarsi 1–4 lobed, penultimate tarsomere greatly reduced; pretarsal claws small, simple (Figure 1G and Figure S2F).

Abdomen with five free ventrites; ventrite 1 slightly longer than 2, without postcoxal lines; intercoxal process poorly developed. Ovipositor longer than wide; gonocoxites with short gonostyli (Figure S2H).

Male unknown.

Cretoparacucujus cycadophilus sp. nov. Cai and Escalona

ZooBank LSID: urn:lsid:zoobank.org:act:377DF250-3F15-4A47-AAD4-EEE71FE3909D.
(Figure 1A, B, D, F, G and Figures S1A–D, F, and 2A, C, D, F, H)

Etymology. The specific epithet is a combination of Greek *kykas* (meaning, cycad) and *philia* (meaning, friendly love or affection).

Material. Holotype, NIGP166883, female; lowermost Cenomanian, from an amber mine located near Noije Bum Village, Tanaing Town, Hukawng Valley, northern Myanmar; deposited in the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing, China.

Locality and Age. The specimen is derived from an amber mine located near Noije Bum, Tanaing, Kachin, Myanmar. The U-Pb dating of zircons from the volcanoclastic matrix yielded an age of 98.79 ± 0.62 million years.

Diagnosis. As for the genus (*vide supra*).

Description. Body elongate, small, ca. 2.0 mm long. Colour light brown, with antennae and apices of mandibles slightly darker. Dorsal surfaces glabrous. Antenna with antennomere 1 elongate, about 2.3 times as long as wide; antennomere 2 distinctly narrower than antennomere 1, about 2.1 times as long as wide; antennomere 3 slightly longer than antennomere 2; antennomere 4 as long as antennomere 2; antennomere 5 about 1.4 times as long as antennomere 4; antennomeres 6–10 almost of same shape and size, each shorter than antennomere 5; antennomere 11 elongate, gradually pointed towards apex. Clypeus apex widely notched medially, sides bidentated. Maxillary palpus elongate, palpomere 2 ca. 0.21 mm long, palpomere 3 ca. 0.22 mm long, palpomere 4 ca. 0.15 mm long. Pronotum transverse, about 0.32 mm long and 0.49 mm wide; punctation fine and sparse, with most punctures separated by about four puncture-diameters. Elytra 1.17 mm long, each about 0.38 mm wide, about 3.6 times as long as pronotum. Gonostylus narrowly cylindrical, sub-apical.

2. Phylogenetic analysis

2.1 Taxon selection

Exemplar taxa for phylogenetic analyses were selected from adult specimens only, because larvae of the fossil genus *Cretoparacucujus* are not known. Exemplars included: (i) one Cretaceous species, *Cretoparacucujus cycadophilus* gen. et sp. nov.; (ii) species belonging to all six extant genera of Boganiidae; (iii) *Eronyxa* sp. (Cleroidea: Trogossitidae: Lophocaterinae) as the outgroup, and (iv) five representatives of closely related cucujoid families.

     The modern boganiids include: *Boganium armstrongi*, *B. malleense*, and *B. medioflavum*; *Afroboganium elmeae* and *A. proprium* (Boganiinae); *Paracucujus rostratus*; *Metacucujus encephalarti*; *Athertonium parvum* and *A. williamsi*; and *Dzumacium caledonicum* (Paracucujinae). The other cucujoid taxa include: *Byturus* sp. (Byturidae), *Ericmodes* sp. (Protocucujidae), *Hobartius* sp. (Hobartiidae), *Pharaxonotha* sp. (Eroytlidae: Pharaxonothinae), and *Protosphindus* sp. (Sphindidae: Protosphindinae).

2.2 Morphological characters used in the analysis

1. Anterior margin of clypeus. 1) without teeth. 2) with small teeth (two teeth; Supplementary Figure 2D, E).
2. Mandible shape of female. 1) short and curved (Figure S2E). 2) long and straight (Figure S2D).
3. Elytral setae. 1) absent (Figure S1A). 2) present.
4. Transverse occipital carina. 1) absent. 2) present (Figure S1D).
5. Median frontal endocarina. 1) absent. 2) meeting frontoclypeal sulcus (Figure S1D). 3) not meeting frontoclypeal sulcus.
6. Antennal insertions. 1) not completely concealed by frontal ridges, at least partly visible in dorsal view. 2) completely concealed from above by frontal ridges (Figure S1D, E).
7. Subantennal groove. 1) absent (Figure S1F, G). 2) present between compound eye and mouth cavity. 3) extending below or behind compound eye.
8. Frontoclypeal sulcus. 1) absent or incomplete. 2) complete, straight or faintly curved (Figure S1D, E). 3) complete, curved, or angulate.
9. Labrum. 1) moderately to well sclerotized and visible for most of its length. 2) more or less membranous and concealed or visible at apex only.
10. Antennal flagellum. 1) filiform, antennomeres more or less equal in width and elongate or subquadrate (Figure S1C). 2) appearing filiform, but with weak 3-articled club. 3) distinctly capitate, with last three antennomeres distinctly wider and longer than preceding antennomeres.
11. Mandibular apex. 1) simple, not subdivided. 2) divided into two or more lobes or sharp teeth.
12. Lateral edge of mandible. 1) without glandular opening. 2) with glandular opening.
13. Incisor edge of mandible. 1) without fixed lobes or teeth. 2) with one simple fixed lobe or tooth. 3) with bifid lobe or two or more fixed lobes, teeth, or serrations.
14. Dorsal surface of mandible. 1) without cavity. 2) with glabrous cavity. 3) with setose cavity (Figure S1D, E).
15. Dorsal surface of mandible. 1) without tubercle or ridge. 2) with tubercle fitting into depression on lateral edge of clypeus.
16. Prostheca. 1) well-developed, usually membranous and fringed with setae. 2) reduced, often only a fringe of short setae.
17. Lacinia. 1) moderately long, extending beyond middle of galea, not much narrower than galea. 2) moderately long, extending beyond middle of galea, but much narrower than galea. 3) short, not extending beyond middle of galea.
18. Lacinia. 1) with bidentate apical or subapical uncus. 2) with unidentate apical or subapical

uncus. 3) without uncus.

19. Apical maxillary palpomere. 1) cylindrical (Figure S2A, B). 2) fusiform (narrowed at both ends) or conical (narrowed apically). 3) slightly expanded and truncate apically.

20. Apical labial palpomere. 1) more or less cylindrical (Figure S1F, G). 2) fusiform (narrowed at both ends) or conical (narrowed apically). 3) slightly expanded and truncate apically.

21. Tentorium. 1) with anterior arms slender, not mesally expanded. 2) with anterior arms expanded mesally to form laminatentoria, sometimes meeting at midline.

22. Corpotentorial bridge. 1) straight. 2) arched. 3) with anterior process.

23. Lateral cervical sclerites on each side. 1) at least 0.15 times as long as head width behind compound eyes, often subdivided. 2) less than 0.15 times as long as head width behind compound eyes, not subdivided. 3) absent.

24. Anterolateral portion of pronotum. 1) without glandular pore or callosity. 2) with glandular pore or pores only. 3) with glandular pore or pores on callosity.

25. Anterior pronotal angles as viewed from above. 1) not produced forward, rounded or angulate. 2) produced forward and broadly rounded.

26. Posterior, sub-basal portion of pronotal disc. 1) without paired impressions. 2) with pair of broad, shallow impressions. 3) with pair of small, well defined impressions or pits.

27. Lateral portion of prosternum in front of middle of procoxal cavity. 1) less than 0.5 times as long as mid-length of cavity or coxal base at that point. 2) 0.5 to 2 times as long as mid-length of cavity or coxal base at that point. 3) more than 2 times as long as mid-length of cavity or coxal base at that point.

28. Prosternal process in ventral view. 1) not expanded apically. 2) slightly or gradually expanded apically. 3) distinctly and abruptly expanded apically.

29. Protrochantin. 1) exposed. 2) concealed.

30. Procoxal cavities externally. 1) broadly open. 2) narrowly open. 3) narrowly closed. 4) broadly closed.

31. Procoxal cavities internally. 1) open. 2) closed by slender bar. 3) broadly closed.

32. Procoxal cavities. 1) contiguous or separated by less than 0.25 times shortest diameter of procoxal cavity. 2) separated by 0.25 to 0.75 times shortest diameter of procoxal cavity. 3) separated by more than 0.75 times shortest diameter of procoxal cavity.

33. Anterolateral edge of procoxal cavity. 1) without narrow extension. 2) with narrow extension.

34. Postcoxal projections of hypomeron. 1) absent or very short and usually rounded, angulate or truncate. 2) moderately long and acute. 3) meeting prosternal process. 4) meeting opposing postcoxal projection.

35. Anterior edge of mesoscutellar shield. 1) not or only slightly or gradually elevated. 2) distinctly and abruptly elevated.

36. Elytral punctures. 1) irregularly aligned or with a few vaguely defined rows. 2) with more than five distinct puncture rows or striae.

37. Elytral scutellary striole. 1) present. 2) absent.

38. Elytral apices, when closed. 1) without exposed sutural flange. 2) with exposed sutural flange.

39. Elytral epipleuron. 1) complete to apex. 2) not complete to apex.

40. Paired procoxal rests on anterior edge of mesoventrite. 1) absent. 2) present.

41. Mesotrochantin. 1) exposed. 2) concealed or absent.

42. Mesocoxal cavities. 1) contiguous or separated by less than 0.25 times shortest diameter of coxal cavity. 2) separated by 0.25 to 0.75 times shortest diameter of coxal cavity. 3) separated by more than 0.75 times shortest diameter of coxal cavity.

43. Mesocoxal cavity bordered by: 1) mesoventrite, mesanepisternum, mesepimeron, metanepisternum and metaventrite. 2) mesoventrite, mesanepisternum, mesepimeron and metaventrite. 3) mesoventrite, mesepimeron and metaventrite. 4) mesoventrite and metaventrite.

44. Exposed portions of metacoxae. 1) extending laterally to meet elytra or sides of body. 2) not

extending laterally to meet elytra or sides of body.

45. Metendosternite (stalk). 1) at least as long as wide. 2) shorter than wide or absent.
46. Metendosternite (laminae). 1) well developed and broad. 2) moderately well developed but narrow. 3) absent or only barely protruding from arms.
47. Hind wing (post-radial sclerite). 1) absent. 2) present.
48. Hind wing (radial cell). 1) complete (Figure S2C). 2) greatly reduced or absent.
49. Hind wing (binding patch anteriad MP3+4). 1) absent. 2) present.
50. Tarsal formula. 1) 5-5-5. 2) 5-5-5 in female and 5-5-4 in male. 3) 5-5-5 in female and 4-5-5 in male.
51. Penultimate mesotarsomere. 1) not much shorter than preceding tarsomere and not enveloped by its ventral lobe. 2) much shorter than preceding tarsomere but not enclosed by its ventral lobe. 3) simple, much shorter than preceding tarsomere and encompassed by its ventral lobe (Figure S2E, F, G).
52. Paired posterosublateral abdominal glands. 1) absent. 2) present on ventrites 1–4. 3) present on ventrites 1–5.
53. Anterior edge of sternite VIII in male. 1) without median strut. 2) with median strut.
54. Anteroventral edge of segment IX in male. 1) with broadly rounded, broadly truncate or emarginate subgenital plate. 2) with narrowly rounded, narrowly truncate or subacute subgenital plate. 3) with single median strut or spiculum gastrale.
55. Posterior edge of sternite IX in male. 1) without projecting mesal lobe. 2) with projecting mesal lobe or tegmenite.
56. Parameres. 1) articulated at base, rarely fused together. 2) fused to phallobase, visible as two projecting lobes or apparently absent.
57. Phallobase. 1) symmetrical. 2) asymmetrical.
58. Anterior edge of phallobase or tegmen. 1) without struts. 2) with single apodeme or strut. 3) with single apodeme and opposing paired struts.
59. Penis. 1) not divided into basal and apical sections. 2) divided into basal and apical sections.
60. Anterior edge of penis. 1) without or with single strut. 2) with paired struts.
61. Anterior edge of sternite VIII in female. 1) with fixed median strut (spiculum ventrale), usually forked at base. 2) with articulated median strut (spiculum ventrale) never forked at base.
62. Paraproct. 1) shorter than gonocoxite. 2) not more than 1.5 times as long as gonocoxite. 3) more than 1.5 times as long as gonocoxite.
63. Gonostyli. 1) attached apically. 2) attached subapically (Figure S2H, I).