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A specialized prey-capture apparatus in mid-Cretaceous rove beetles

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The hyperdiverse rove beetle genus *Stenus* possess one of the most specialized prey-capture structures known from extant arthropods [1,2]. The harpoon-like apparatus is formed by an elongated protrusible labium with terminal sticky cushions (Figure 1A,B) and a haemolymph pressure mechanism that enables the beetle to catch fast-fleeing prey within a few milliseconds [1–5]. Living in various microhabitats including on vegetation, extant *Stenus* species prey on fast-fleeing springtails (Collembola) and other small animals [1–3]. They are observed to secrete an adhesive during prey capture [5], and the morphology of the cushions accounts for variation in adhesive performance among species and between prey types [1,4,5]. Fossils with an exposed prey-capture apparatus are exceedingly rare, the oldest being from Eocene Baltic amber (approximately 45 million years ago [mya]) [6]. Mesozoic stenines are therefore significant for elucidating the early evolution of the group and have been documented from middle–late Cretaceous deposits in France, Myanmar

and Russia [6]. However, direct evidence of the exposed prey-capture apparatus is lacking. Here we confirm the Mesozoic antiquity of this key innovation in two species of the extinct genus *Festenus* recently described [6] from mid-Cretaceous Burmese amber (approximately 99 mya) and representing one of the earliest members of Steninae. We therefore provide critical information about the origin and early evolution of both the novel predatory structure and of the subfamily Steninae.

Steninae include three extant genera, the widespread *Stenus* (~3000 species; one of the most species-rich animal genera), the Holarctic *Dianous* (~220 species), and a new genus from Australia with three new species [7]. We examined over 120 individuals of Steninae among 23,000 fossiliferous Burmese amber pieces in our collection, only two of which preserved an exposed prey-capture apparatus (Supplemental Information). One is *Festenus gracilis* (NIGP168835; Figure 1C) [6] as supported by the body shape and the characteristic bicolored elytra, and the other represents a new species of *Festenus* (NIGP168836; Figure 1F). The prey-capture apparatus in NIGP168835 is partly protruded showing completely preserved apical adhesive paraglossae (Figure 1D), whereas in NIGP168836 it is disassociated from the head and the adhesive paraglossae are not preserved (Figure 1F–H).

As in *Stenus* and the Australian genus [1,7], the prey-capture apparatus in NIGP168835 is formed by the extended labium (Figure 1D). The labial palpi are 3-segmented with an elongate palpomere 1, dilated sparsely setose palpomere 2 and acicular palpomere 3. The prementum is slightly widened toward its apex, bearing a pair of anterolateral paraglossae (Figure 1D). As in most extant *Stenus* (e.g., *S. comma*), these paraglossae form ellipsoid cushions with dense terminal ramifications (Figure 1D, E) that increase the chance of successful adhesion [4–5]. A shiny film appears to cover these ramifications (Figure 1E), suggesting that the outgrowths were probably covered in adhesive secretion. The paraglossae display a generalized form (as in *S. comma*), which may have been modified in various ways during the evolutionary history of stenines [4]. The visible part of the prementum is short, but it is only partly extended and the connecting tube still hidden

within the head. In NIGP168836, however, the prey-capture apparatus is disarticulated from the mouthparts and although only residual parts of the paraglossae were preserved (Figure 1G, H), the prementum is approximately 2.0 x the length of the head, similar to many extant species of *Stenus* (e.g., *S. bimaculatus*, *S. bipunctatus* and *S. comma*). Thus, because the connecting tube is as long as the prementum, in NIGP168836 the adhesive paraglossae could be hurled a maximum distance of approximately 4.0 x the head length to catch prey. Collectively, the comparison of these fossils with extant species confirms that the labium of *Festenus* species was protrusible and could be withdrawn back into the head when not in use. Therefore, the fossil species with large modified cushions and possible adhesive secretion may represent an active and efficient predator in the ancient forest, as also suggested by its globular eyes and long slender legs. Springtails in Burmese amber are very abundant and diverse, and may have been utilized by at least a coeval lineage of specialized ant-like stone beetles (Staphylinidae: Scydmaeninae) [8]. Despite the effective escape mechanism of springtails [8], Cretaceous stenines were likely able to catch them and other microarthropods using their sticky harpoons.

Our discovery of direct evidence of a protrusible prey-capture apparatus in the earliest known stenine beetles from the mid-Cretaceous illuminates their early evolution and confirms an ancient origin for their derived predatory behavior. Among extant stenines, the sticky harpoon mechanism is well developed in *Stenus* and the Australian genus (Supplemental Information), but absent in *Dianous* [1,7]. It is challenging to reconstruct the phylogenetic tree of Steninae [6,9] due to insufficient sampling of this hyperdiverse group, which usually defies species-level identification. Molecular phylogenetic studies of selected species of *Stenus* and *Dianous* indicated an evolutionary origin of *Dianous* within *Stenus*, suggesting a secondary loss of the harpoon apparatus [9]. This hypothesis is further reinforced by another phylogenetic result that the Australian genus, which possesses a prey-capture apparatus homologous to that of *Stenus*, is a sister group to *Stenus* + *Dianous* (all sampled genera represented by one species) [10]. The presence of a protrusible

prey-capture apparatus has been considered the most prominent apomorphic character for *Stenus* [1], but this assumption is not supported by recent discoveries of extant and extinct Steninae. Additionally, the ellipsoid cushions can be found in *Festenus*, the Australian genus and most studied *Stenus* species, suggesting that this common form is the likely ground plan for structure and function of the adhesive paraglossae in Steninae.

Steninae is recovered as the sister group to Euaesthetinae in morphological and molecular phylogenies [6,7,10]. Interestingly, the euaesthetine genus *Tyrannomastax* (Madagascar) has an elongate protrusible labium possibly also functioning as a prey capture apparatus [7], but it is short and the paraglossae differently modified. It is possible that this similar elongation of the labium resulted from a convergent lifestyle in the forest litter layer.

SUPPLEMENTAL INFORMATION

Supplemental Information includes experimental methods, one figure and additional references, and can be found with this article online at https//xxx.

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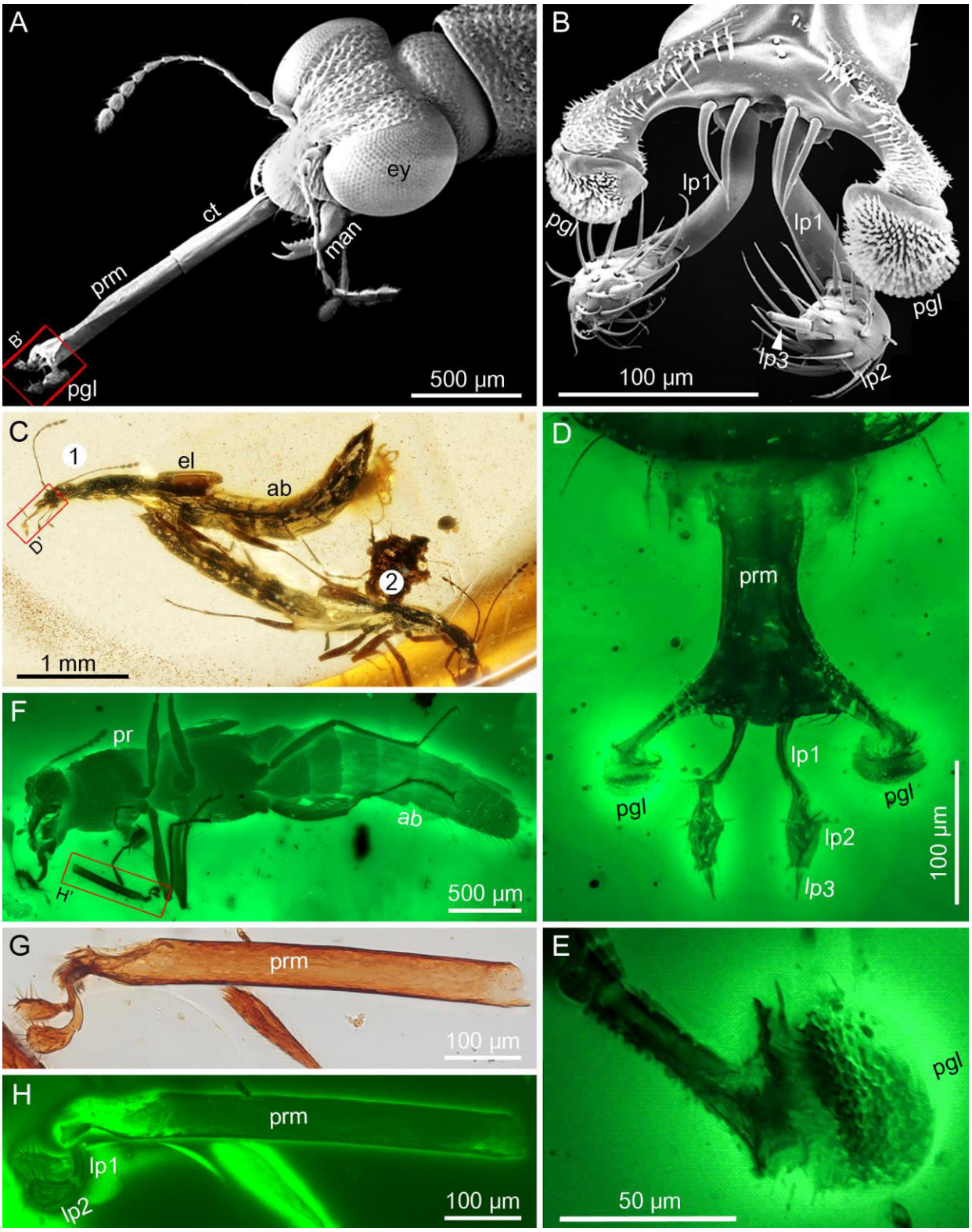
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Figure 1. Specialized prey-capture apparatus in extant and mid-Cretaceous stenine rove beetles.

(A) Prey-capture apparatus of extant *Stenus comma*, scanning electron microscopic image, adapted from Bauer and Pfeiffer [3] and reproduced with permission. (B) Enlargement of (A), showing details of sticky cushions (paraglossae). (C) Two individuals of *Festenus gracilis* in mid-Cretaceous amber from Myanmar; the upper one (labelled with '1'; NIGP168835) with prey-capture apparatus partly exposed, under reflected light. (D) Frontal view of NIGP168835, showing prey-capture apparatus, under epifluorescence. (E) Lateral view of paraglossa, with outgrowths apparently immersed within adhesive secretion, under epifluorescence. (F) Ventral view of *Festenus* sp. (NIGP168836), showing disarticulated prey-capture apparatus and maxillary palpi, under epifluorescence. (G) Enlargement of (F), showing prementum and labial palpi; (H) Same as (G), under confocal microscopy, showing the residual parts of paraglossae. Abbreviations: ab, abdomen; ct, membranous connecting tube; el, elytron; ey, eye; lp1–3, labial palpomeres 1–3; man, mandible; pgl, paraglossa; pr, pronotum; prm, prementum.