

Correspondence

Non-sexual abdominal appendages in adult insects challenge a 300 million year old bauplan

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Despite their enormous diversity, the bauplan of adult winged insects (pterygotes) is remarkably conservative since the Lower Devonian: a five-segmented head, a three-segmented thorax with three pairs of walking legs and an eleven-segmented abdomen without any non-sexual appendages [1,2]. The only known exceptions are the abdominal appendages of adult male sepsid flies on the fourth segment; however, these are also used as copulatory organs and are supposedly maintained through sexual selection [3]. Here, we report a rod-like paired appendage from the third and fourth abdominal segments in adults of the Southeast-Asian Hemiptera taxon Bennini (Figure 1A,B; Supplemental information). These are fully muscled, innervated, and movable and bear highly organized sensory and secretory units. The appendages, termed LASSO (lateral abdominal sensory and secretory organs), are consistent in topology and structure in all species studied and not sexually dimorphic. The existence of these non-sexual abdominal appendages reveals the potential of the 300 million year old conserved bauplan of insects.

Pregenital abdominal appendages found in non-pterygote taxa (well-known examples are the jumping apparatus in Collembola and the coxal styli in Archaeognatha) or in immature

pterygotes (e.g., the tracheobranchial appendages in mayfly nymphs, the tracheal gills of larval gyridid beetles and those of neuropterids such as *Corydalid*) usually serve functions other than sexual, i.e., they are either respiratory or locomotory.

As a first step to elucidate what drove the evolution of non-sexual abdominal appendages in Bennini, we investigated topology and anatomy of the appendages. Each LASSO consists of a proximal slender, narrow, cylindrical process (the ‘rod’) and an apical dilation (the ‘cup’) (Figure 1C). The long rod-like appendage is formed in part by the epipleurite of segment IV and in part by the membrane connecting it with abdominal sternite IV [4]. Cephalically the proximal margin of epipleurite IV (the rod), a rectangular, medially slightly bent sclerite (presumably the epipleurite of abdominal segment III), shields the membranous connection between sternite IV and epipleurite IV (terminology follows Sweet [5]). From the anterior margin of abdominal sternite IV, an apodeme connects with the proximal end of epipleurite IV, presumably providing muscular attachment. The rod is caudally concave, with the concave part membranous while the cup is strongly sclerotized throughout. The distal dilated part, the cup, usually carries a whitish, apparently waxy ‘hood’ which encloses a hollow space (Figure 1D,E; Supplemental information). When this hood is removed, it becomes obvious that the cup is apically slightly concave, in top view horseshoe-shaped, and framed by a rounded fringe except for a small area at the narrower side: from there a broad obtuse ridge extends medially over ca. three quarters of the area enclosed by the outer fringe (Supplemental information). Around the broad median ridge and parallel to the outer fringe runs a row of ca. 70 short peg-like sensillae. Between them and the median ridge a series of narrow sharp radial ridges is located. In all Bennini species examined, two long, filiform setae arise from distinct, subglobular sockets (Figure 1E; Supplemental information), situated on the median ridge, in a row between centre and exterior fringe. As no obvious wax glands were otherwise observed at the margin of the distal cup-shaped dilation, it seems evident that the waxy hood is the product of the gland underneath the observed filament. The mode of construction of

the hood, however, remains entirely unknown. A 3D reconstruction based on micro-CT images and histological sections (Figure 1C; Supplemental information) revealed that the rod contains muscular and nervous tissue as well as a tracheal branch which opens with a spiracle into the membranous fold of the rod. The cup accommodates two distinct areas of glandular tissue: one flat, horseshoe-shaped, situated directly underneath the cup’s lid, the other elongate and adjacent to the cup’s exterior wall. The horseshoe-shaped gland is likely to produce the wax that eventually forms the hood. The cup also contains a short, sturdy muscle which inserts on the inside of the lid and at the lateral wall of the cup; its contractions probably aid in exuding the wax.

The LASSOs are fully developed as free appendages, and thus functional, only in the adult stage. Fourth and fifth instar nymphs display *anlagen* of the LASSO which are visible through the larval epidermis but do not protrude externally (Figure 1F–H; Supplemental information). The analysis of micro-CT data of the nymphal appendage *anlage* clearly reveals the presence of a spiracle (tracheal opening) at about midlength of the rod (Figure 1I; Supplemental information), thus corroborating the assumption that it is the derivative of an epipleurite and pleural membrane. During the development of the adult configuration the spiracle subsides into the concave membranous part of the rod.

The existence of the LASSO provides an opportunity for testing current concepts of homology, which addresses the complex relationships between structural and developmental hierarchy levels [6,7]. The topology of body parts making up the LASSO and its complex structure shows that it is not a serial homologue of an abdominal limb, but an evolutionary novelty. Our observation that the LASSO is made up of derivatives of two consecutive abdominal segments (epipleurites of segments III and IV as well as pleural membrane of segment IV as is evident by the position of the spiracle) challenges the one-segment-one-appendage paradigm [8].

Strong natural selection can be assumed as the appendage is highly complex, yet uniform across Bennini and not sexually dimorphic. The behavioral or ecological context and

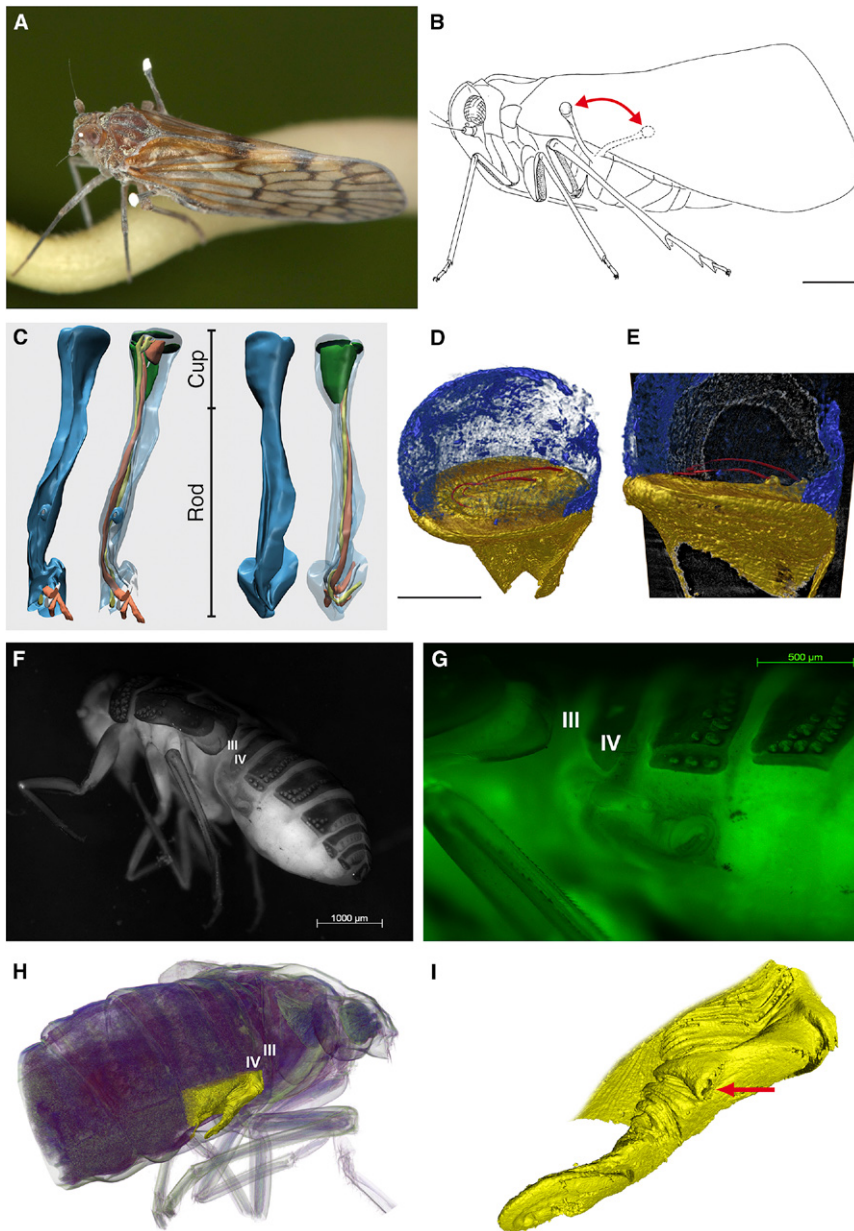


Figure 1. Structure of the LASSO in Bennini adults and nymphs. (A) Adult Bennini male in usual pose, i.e., with LASSO directed dorsoanteriorly (*Celebenna thomara*) [9]. (B) Sketch of adult Bennini female, demonstrating the degree of LASSO movement. Scale bar: 1 mm. (C) 3D reconstruction of LASSO appendage from histological slides (male of *Bennaria damisa*) [10]. *Left:* ventral aspect; blue: integument (note the concave membranous portion with spiracle in proximal (here: lower) half); red: muscle tissue; yellow: nerve; white: trachea; green: glandular tissue. Note the short strong muscle in the cup-unit. *Right:* dorsal aspect. Same color coding. Note the two glands in the cup-unit. (D) External anatomy of the LASSO's cup unit (Bennini *gen. spec.*: Vietnam). Distal surface of cup-unit, slightly tilted and rotated 90°. 3D reconstruction from micro-CT images, wax hood partially transparent. Blue: wax hood; gold: integument; red: filiform sensillae. Scale bar: 100 μ m. (E) Same as (D), tilted backwards; color coding as in (D), orthoslice underlying volume rendered image. Note the well-defined inner surface of the wax hood. For an animation see Supplemental information. (F–I) LASSO *anlage* in Bennini nymphs (*Bennaria spec.*, Bohol Island, Philippines). (F) Last (V) instar nymph, overview, left lateral aspect. Note LASSO *anlage* as dark area at base of the abdomen. III, IV: abdominal segments III and IV. (G) Same as (F), detail, autofluorescence-enhanced image: LASSO *anlage* visible under nymphal integument. (H) Last (V) instar nymph, right lateral aspect, volume-rendered 3D reconstruction from micro-CT images (violet/green) with LASSO *anlage* displayed as isosurface (bright yellow). (I) Same aspect as in (H), detail. LASSO *anlage* enlarged, isosurface. Note the prominent position of the spiracle (arrow) (see text for further explanation). For an animation of the 3D reconstruction see supplemental information.

the nature of the stimuli processed by the LASSO are not yet clear; however, they do not appear to play a prominent role in intraspecific communication [9]. The distance between two sensory units is a well-known solution for directional signal perception. It thus is conceivable that the LASSO might be an enemy-detection device.

Supplemental Information

Supplemental Information including experimental procedures, three figures two movies and an interactive PDF can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2013.11.040>.

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