Structural mouthpart interaction evolved already in the earliest lineages of insects

Hexapoda (insects in the broad sense) evolved an astonishing diversity of mouthparts tailored to use different resources of food. For example, dragonflies and crickets use biting-chewing motions of their mandibles to chop food particles, true bugs evolved piercing-sucking mouthparts to suck fluids from plants, flies evolved sponging mouthparts, and moths and butterflies evolved the unique proboscis to siphon mostly nectar of flowers [1,2]. Although mouthparts *functionally* interact in nearly all insects to process food, many winged insects evolved a *structural* interaction resulting in the formation of new mouthpart types [1]. Corresponding structural changes are radical and morphologically very different to each other, a comparable diversity and complexity of mouthparts is not present in any other arthropod group. It is unclear when this major trend in insect evolution – structural mouthpart interaction (SMI) – evolved for the first time.

In order to assess this question, we used synchrotron μCT (SR-μCT) imaging setups to investigate the mouthpart anatomy of Collembola and Diplura - ancient, tiny, soil-living insects. High resolution SR-μCT was done at the beamline **BL47XU** using a stable beam energy of 8 keV in absorption-contrast mode. The tomography system consists of a full field X-ray microscope with Fresnel zone plate optics. The field of view and the effective pixel size are 0.11 mm × 0.11 mm and 82.6 nm × 82.6 nm, respectively.

Collembolan and dipluran mouthparts show a specific and unusual type of SMI, based on an articulatory point between the mandible and the maxilla [3]. In Diplura, the contact point is formed by an articulatory stud of the maxillary stipes ("STST"; Fig. 1(a,b)). This stud is a short, upraised prominence, and is supported by two internal stipital strengthening ridges. The dorsal ridge originates at the base of the stud and extends along the inner stipital wall to fuse with the ventral stipital ridge [3]. The tip of the stud is in contact with a slight concavity at the posterior outer wall of the mandible [3].

The newly found SMI in Collembola also is composed of a stipital stud (STST) that originates at the median anterior (dorsal) wall of the stipes (Fig. 1(c,d)). As in Diplura, this stud is supported by two strengthening ridges, one spanning from the base of the stud to the opposite side of the maxillary stipes where it fuses with the second strengthening ridge [3].

The stipital studs of the maxillae in Collembola

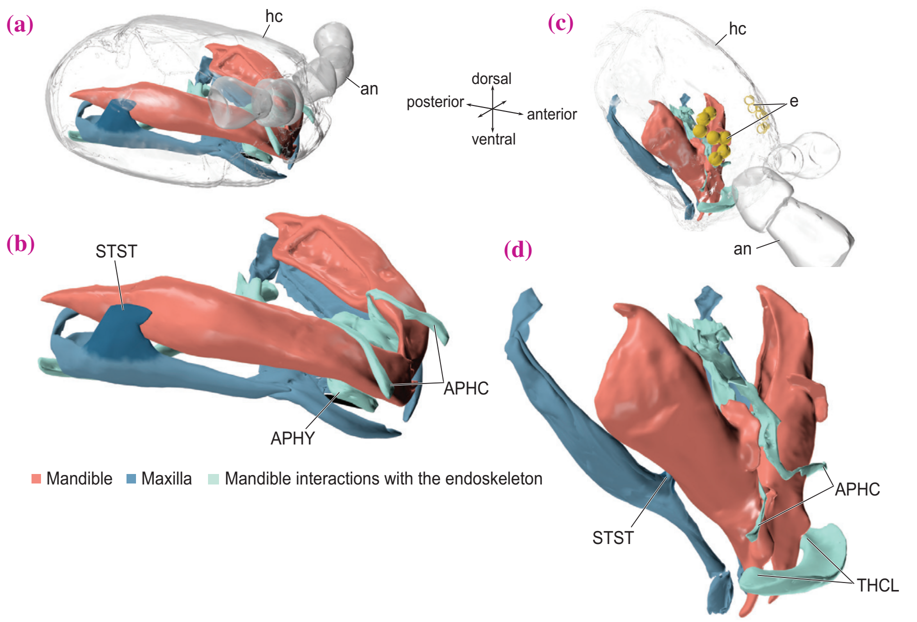

Fig. 1. Overview of dipluran and collembolan mouthpart organisation illustrated with 3D reconstructions of SR-μCT data. **(a)** Dorsolateral view of a dipluran to indicate the location of the mandible-maxilla hypopharynx complex in the head capsule. **(b)** Same as (a) with head capsule removed. Note the stipital stud (STST) embracing the posterior part of the mandible and the apodemes of head capsule (APHC) and hypopharynx (APHY) embracing the anterior inner part of the mandible. **(c)** Dorsolateral overview of the mouthpart location within the collembolan head. **(d)** Same as in (c) with head capsule removed. Note the stipital stud (STST) contacting the mandible to prevent posterior sheering out of the mandible. The thickening of the clypeus (THCL) prevents lateral sheering out of the mandibles, while the apodemes of head capsule (APHC) and hypopharynx (APHY, not shown) embrace the anterior inner part of the mandibles just like in Diplura. Additional abbreviations: an, antenna; e, eye; hc, head capsule.

and Diplura are most probably homologous [3]. In both groups, these articulatory studs are located on the dorsal side of the stipes and are supported by a conspicuous configuration of two strengthening ridges at the inner side of the stipes that show the same arrangement. These ridges reinforce the stipital studs to counter loads imposed on the maxilla during mandible movement. The two modes of mandible-maxilla interaction in Collembola and Diplura are thus the first examples of a structural mouthpart interaction in ancestrally wingless insects.

This result has important implications for our understanding of insect mouthpart evolution (Fig. 2). The presence of a homologous SMI in Collembola and Diplura implies the presence of this basic principle in stemgroup representatives of the entire Hexapoda (insects in the widest sense; Fig. 2). It remains unclear until which point SMIs are exhibited in the stemline of early insects before this principle occurs again in more derived winged insects (Fig. 2). However, the biting-chewing mouthpart type with mandibles connected to the head via articulations, as shown in jumping bristletails, can no longer be attributed as the plesiomorphic condition in insects [3]. Rather, we suggest that a "light" form of entognathy with an enlarged subgenal area, probably overgrowing the mouthparts laterally to some extend, might be ancestral. In this scenario, the mouthparts probably already interacted with each other due to some form of structural coupling. Exposed (Ectognathous), structurally uncoupled mouthparts are a derived groundplan feature of silverfish and winged insects.

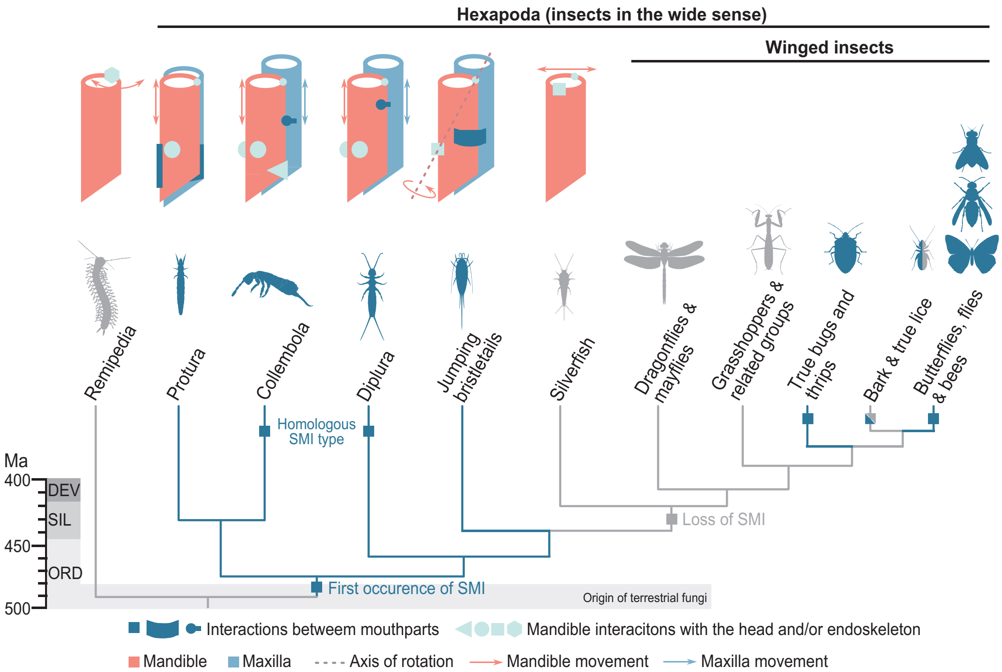

Fig. 2. Evolution of structural mouthpart interactions in insects mapped on a transcriptome based phylogenetic tree and divergence time estimate [4]. Shapes of mouthparts and articulation points are shown as simplified models, mandible and maxilla movements with red and blue arrows respectively. Note that the same forms of interaction points indicate putative homologous structures across taxa. Also note the differing SMI solutions to achieve mandible stabilization during food uptake in Protura, Collembola, Diplura and jumping bristletails. The common ancestor of insects (Hexapoda) most probably already possessed a mandible-maxilla SMI. The occurrence of SMI can be correlated with the origin of fungi during the Ordovician, one of the principal diets of recent Collembola and Protura. In bristletails, an anterior mandibular articulation (dicondyly) evolved, which made other supporting or stabilizing structures obsolete during the further evolution of dicondyly in silverfish and winged insects. Structural mouthpart interaction again occurs in extant true bugs and thrips, lice, and large parts of bees, butterflies and flies, where parts of the mouthparts function as guiding structures for other mouthparts or even fuse together. The occurrence of these new mouthpart types again can be correlated with the evolution of food sources such as diverse plant and animal fluids.

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References

[1] D. Grimaldi and M.S. Engel: Evolution of the Insects (Cambridge University Press, 2005)
[2] C.C. Labandeira: Annu. Rev. Ecol. Syst. **28** (1997) 153.
[3] *A. Blanke, P.T. Rühr, R. Mokso, P. Villanueva, F. Wilde, M. Stampanoni, K. Uesugi, R. Machida, B. Misof: Proc. R. Soc. B* ***282*** *(2015) 20151033.*
[4] B. Misof *et al.*: Science **346** (2014) 763.