

# The first tunicate from the Early Cambrian of South China

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Here we report the discovery of eight specimens of an Early Cambrian fossil tunicate *Shankouclava* near Kunming (South China). The tunicate identity of this organism is supported by the presence of a large and perforated branchial basket, a sac-like peri-pharyngeal atrium, an oral siphon with apparent oral tentacles at the basal end of the siphonal chamber, perhaps a dorsal atrial pore, and an elongated endostyle on the mid-ventral floor of the pharynx. As in most modern tunicates, the gut is simple and U-shaped, and is connected with posterior end of the pharynx at one end and with an atrial siphon at the other, anal end. *Shankouclava* differs from *Cheungkongella*, which was previously called a tunicate. Based on new, more complete “*Cheungkongella*” specimens that show branching tentacles, this form may be a lophophorate, and in any case is not a tunicate.

Tunicates of the subphylum Urochordata (1) represent the most basal branch of known chordates, presumably having arisen  $\approx 543$  million years ago in the “Cambrian explosion,” or even earlier (2). However, no reliable fossil tunicates have ever been reported. The Cambrian fossil *Cheungkongella* was reported to be a tunicate (3), but our observations indicate that its supposed pharynx contains the entire gut, and that this animal had long tentacles (a distinctly nontunicate feature). Another so-called tunicate fossil, *Jaekelocarpus*, from the Carboniferous (4), is covered with large calcite plates instead of a tunic, and its supposed branchial bars have an unusual coronal orientation, so a tunicate identity is unlikely. Here we report a previously undescribed fossil tunicate that is  $\approx 520$  million years old.

*Shankouclava shankouense* is from the Lower Cambrian Maotianshan Shale at Shankou village, Anning, near Kunming (South China). All eight known specimens are from an  $\approx 2$ -cm-thick mud bed. Like most of the fossiliferous layers of the Maotianshan Shale containing soft-bodied organisms, this one represents a microturbidite deposit from the distal part of a shallow marine delta environment, which comprises a lower, graded unit with abundant algal remains and an upper, homogeneous mud unit (5–7). Although most of the other soft-bodied fossils in this and other layers are yellowish in color, the *Shankouclava* specimens are black.

Each of the eight specimens occurs alone and therefore the animal was likely solitary and not colonial. *Shankouclava* was soft-bodied and sac-like, though elongated and pointed proximally (Fig. 1 *B* and *E*; see also Fig. 3). Body lengths of the individuals, estimated from the specimens, range from 2 to 4 cm. Like some modern tunicates, such as *Clavelina*, it had stolons at the proximal end (Fig. 1 *G*), indicating it was sessile and rooted to the ocean floor by these attaching structures.

*Shankouclava* is covered by a grayish organic test, which may be interpreted as a tunic as in the extant tunicates. This tunic was relatively thick, but is incompletely preserved in most specimens; beneath the tunic (at a deeper bedding plane) lies an organic layer comparable to the mantle of extant tunicates (Figs. 1 *F* and *H* and 2 *D* and *E*). In one informative specimen (Fig. 1 *F*), the mantle is seen external to both the left and the right sets of branchial bars, indicating that it extended around the whole body like a true tunicate mantle. The body was

bilaterally symmetrical and club-shaped, and it is divided into a barrel-shaped anterior part and an elongated, triangular, posterior part, which is called the “abdomen” (8–10). The anterior part, which occupies more than half the length of the body, is dominated by a large pharyngeal basket, whose walls are formed by numerous transversely oriented rods interpreted as branchial bars. These bars are not quite straight, as the anterior ones are weakly >>>-shaped and the posterior ones slightly <<<-shaped (Figs. 1 *A*, *B*, *F*, and *H*, and 2). The total number of branchial bars is  $\approx 30$  (Fig. 1 *F*). The detailed anatomy of the branchial bars is often obscured by the overlying tunic or mantle, but in the holotype the bars branch to form a network with rectangular openings (Figs. 1 *A* and *C* and 2 *A*), here interpreted as branchial slits (stigmata) resembling those of living tunicates. Fig. 1 *H* shows another, laterally compacted, specimen, displaying both the right and left pharyngeal walls and a larger, compacted branchial basket between the walls. As in modern tunicates, the pharynx in *Shankouclava* is of large diameter, occupying the greater part of the anterior region of the body and opening posteriorly into a tubular gut. In a sublaterally compacted specimen that shows some of the ventral body (Figs. 1 *E* and 2 *C*), there is an elongated strip extending along the mid-ventral part of the pharynx, here interpreted as an endostyle (6–8). At the distal (“cranial”) end of the animal is a siphon-like structure, located subventrally (Figs. 1 *A–D* and 2 *A* and *B*). This structure resembles the tunicate oral siphon and likewise contains a siphonal chamber with several tentacle-like structures at its base, each  $\approx 0.3$  mm long and 0.2 mm wide. Also as in modern tunicates, the pharyngeal basket is surrounded by a sac-like peripharyngeal chamber, called an “atrium” (8–10) (Figs. 1 *E* and *F* and 2 *C* and *D*). The atrium opens to the outside by a dorsal notched structure, called the “atrial pore” (Fig. 1 *E*). This structure is not clearly visible. It lies above the posterior pharynx about two-thirds of the way back. The postpharyngeal gut loops cranially from the abdomen (see below), and its last part is in the pharyngeal roof, terminating near the atrial pore (Figs. 1 *E* and 2 *C*). In another specimen, an anus is clearly seen in this position, although this latter specimen shows no atrial pore (Figs. 1 *B* and 2 *B*). The abdomen, separated from the body’s anterior region by a notched constriction, is an elongated structure, narrowing proximally (Fig. 1 *B*, *E*, and *H*). A U-shaped tube interpreted as the gut is visible in the abdomen in laterally compacted specimens (Figs. 1 *A*, *B*, *E*, and *H* and 2 *A–C* and *E*). The anterior gut, also called an esophagus, extends posteriorly from the pharynx to near the proximal end of the body and then bends into a loop. The gut in this bend area (Figs. 1 *A* and *E* and 2 *A* and *C*), interpreted as a stomach, is slightly wider than the rest. The posterior gut, also called the intestine, extends anteriorly to end on the pharyngeal roof near the atrial pore, as already described (Figs. 1 *E*, 2 *C*, and 3). The superficial dorsal wall of three specimens shows a series of serial transverse bands extending from the posterior pharynx

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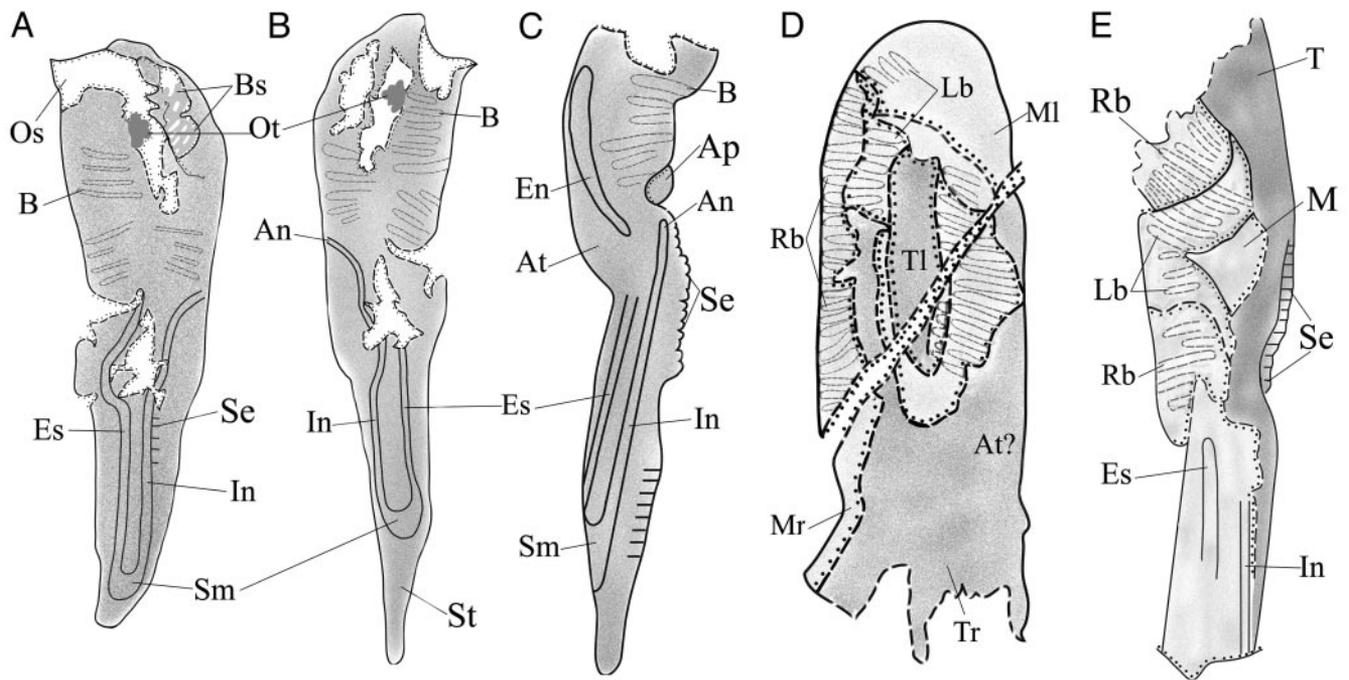
**Fig. 1.** (A–H) Lower Cambrian tunicate *Shankouclava shankouense* gen. et sp. nov. from the Maotianshan Shale, at Shankou Village, Anning, near Kunming. (A and B) Part and counterpart of holotype (SK01001), showing an oral siphon with oral tentacles at its base, a U-shaped gut, body segments, branchial bars and branchial slits. (C and D) Enlargement of A and B, respectively, showing details of the oral siphon with oral tentacles at its base. (E) A laterally to ventro-laterally compacted specimen (SK01002) showing a strip-like endostyle along the ventral part of the pharynx, a not clearly visible atrial pore dorsally, body segments, and U-shaped gut. (F) A specimen showing a complete pharynx (SK01004) with branchial bars. (G) The posterior part of a specimen (SK01005) with attaching stolons. (H) A nearly complete specimen (SK01003), showing both sides of the pharyngeal wall with the overlying mantle and tunic. (I–K) Lower Cambrian lophophorate *Phlogites* (= *Cheungkongella*) from the Maotianshan Shale, at Ercai Village, Haikou, near Kunming. (I and J) Part and counterpart of a laterally compacted specimen (EC01001a,b), showing a large, darkly stained body coelom containing the coiled gut, and a stalk attaching to a trilobite fragment. (K) A specimen (EC01002) with well preserved branched arms on distal margin of calyx. A, arms; An, anus; Ap, possible atrial pore; At, atrium; B, branchial bars; Bc, body coelom; Bs, branchial slits; En, endostyle; Es, esophagus; In, intestine; Lb, left set of branchial bars; M, mantle; ML, left mantle; Mo, mouth; Mr, right mantle; Os, oral siphon; Ot, oral tentacle; Ph, pharynx; Rb, right set of branchial bars; S, stolons; Se, body segments; Sm, stomach; St, stalk; Tl, tunic on left side; Tr, tunic on right side. (Scale bars, 5 mm in A, B, E, F, and H; 2 mm in C, D, and G; and 10 mm in I–K.)

to almost the posterior end of the abdomen (Figs. 1 A, B, E, and H and 2 A, C, and E). The types of tissue from which these serial ridges were composed cannot be determined, and this feature is not seen in modern tunicates.

#### Phylogeny of *Shankouclava*

The sessile organism *Shankouclava* bears a striking resemblance to extant tunicates in the tunic and mantle, the large

perforated pharyngeal region, with numerous transversely oriented structure/branchial bars, the sac-like peri-pharyngeal atrium, the oral siphon, with what appear to be oral tentacles at its base, the dorsal atrial pore, and the elongated endostyle band on the mid-ventral part of the pharynx. The similarity to single zooids of modern colonial aplousobranch tunicates such as *Clavelina* and *Amaroucium* is particularly close (8–10). As with *Shankouclava*, the body of these animals is slender and



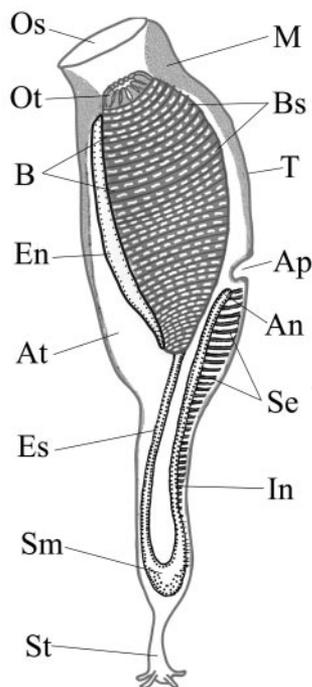
**Fig. 2.** Interpretative drawing of *Shankouclava shankouense* gen. et sp. nov. (A) Diagram of Fig. 1 A. (B) Diagram of Fig. 1 B. (C) Diagram of Fig. 1 E. (D) Diagram of Fig. 2 F. (E) Diagram of Fig. 1 H. Abbreviations are as in Fig. 1.

differentiated longitudinally into two parts of subequal length, and between the two parts is a notched constriction. *Shankouclava* differs from the aplousobranchs in that its atrial pore opens farther posteriorly on the pharyngeal roof.

### Cheungkongella: Another Fossil Tunicate?

Shu *et al.* (3) recently erected the new taxon *Cheungkongella*, based on a single incomplete specimen from the Lower

Cambrian Maotianshan Shale, at Ercai village, Haikou, Kunming, which they interpreted as a tunicate. This animal is shaped like a bulb (calyx) on a stalk, and a dotted, darkly stained area occupying most of the calyx was interpreted as a tunicate pharyngeal basket. This interpretation can be evaluated by considering a coexistent fossil genus from the same locality, *Phlogites*, which is indistinguishable from *Cheungkongella*, and was discovered and described earlier (11). Based on more complete new specimens of *Phlogites* (Fig. 1 I–K), we interpret the darkly stained area in the calyx as a coelom, rather than a pharynx, because in some specimens it is seen to contain the coiled gut (Fig. 1 I and J). This gut is not preserved in most specimens, however, which would explain why the *Cheungkongella* specimen does not show it. Additional evidence indicates that the calyx is not a pharynx: The yellowish dotted pits on the darkly stained film are arranged too irregularly to be the gill openings as claimed (3); rather, they are better understood as irregularities resulting from splitting the rock. In the new specimens, the tubular structure called a “buccal tentacle” by Shu *et al.* (3) is more likely to be a mouth because it opens at the body surface rather than projecting above it. The anus at the end of the intestine is situated on the side of the calyx (Fig. 1 J). This structure was interpreted as a cloacal siphon (3), although it is not funnel-shaped like a tunicate siphon. In the new specimens this hole is shown to be too small to have passed large volumes of ventilatory water. Most importantly, the specimen of *Phlogites* shown in Fig. 1 K clearly displays long branched arms projecting from the calyx. Large tentacles like these do not occur in tunicates. Only incomplete parts of these arms are exposed at the surface of most of the fossils (Fig. 1 I and J), so the total number remains uncertain, with some specimens suggesting three arms and others suggesting five. The *Cheungkongella* specimen does not show any obvious arms, perhaps because its arms broke off or are covered by rock. In summary, the single *Cheungkongella* specimen is concluded to be a specimen of *Phlogites*, and its proposed tunicate affinity is inconsistent with many characteristics.



**Fig. 3.** Lower Cambrian tunicate *Shankouclava* reconstructed. Abbreviations are as in Fig. 1.

The phylogenetic affinities of *Phlogites* (= *Cheungkongella*) remain unclear. The branched circumoral arms and coiled, U-shaped gut with the anus on the side of the body suggest an affinity with the Sipuncula, but all known sipunculans are mobile benthic worms without a stalk, and they have a unique retractable anterior body region called an introvert (1), which *Phlogites* lacks. *Phlogites* also shares a number of features with some echinoderms, including a calyx, stem, branched arms, and U-shaped gut, but it lacks the calcareous endoskeleton and water vascular canals that are typical of echinoderms. It could be allied to the pterobranch hemichordates, but modern pterobranchs are much smaller,  $\approx 1$  mm in size, and they live in a colonies within a protective coenocium (1). Unlike the multiple circumoral arms of *Phlogites*, the arms in pterobranchs are either arrayed in two rows or exist only as a pair, and they do not form a ring around the mouth. We favor the original proposal of Luo and Hu (11) that *Phlogites* was a lophophorate. It resembles lophophorates, especially those of phylum Ectoprocta, in its circumoral arms and U-shaped gut, with the anus lying outside of the crown and arms. However, all modern lophophorates live in a secreted chitinous or calcareous tube or shell, and their digestive tract is rather simple, not coiled. *Phlogites* was a solitary creature and the solitary lifestyle is unusual in modern lophophorates. The only lophophorates that are solitary are brachiopods. The great morphological gap between *Phlogites* and brachiopods indicates that they are not closely related. The solitary, soft-bodied *Phlogites* could belong to an extinct stem group of lophophorates. There is also a close resemblance to entoprocts. However, the anal cone in entoprocts lies within the tentacular crown, whereas in contrast, the anus is in a lateral position in *Phlogites*, so the phylogenetic relation of *Phlogites* to entoprocts is questionable.

#### Phylogenetic Affinities of Calcite-Plated *Jaekelocarpus*

Relationships of a calcite-plated fossil group that include mitrates, known variously as homolazoan echinoderms, calcichordates or “carpoids” (12, 13), are extremely controversial. A Carboniferous mitrate called *Jaekelocarpus* was recently interpreted as a tunicate, on the basis of structures called “lamellae” that may bear gill slits (4). However, these lamella and “gill slits” are horizontal and lack the vertical components of all chordate gill slits and bars. The present discovery of the soft-bodied *Shankouclava*, which is both much older and more tunicate-like in appearance than *Jaekelocarpus*, suggests that this calcite-plated organism was not related to tunicates.

#### Conclusion

Many higher taxa of animals originated during or even before the Cambrian (14–17). This fact has been strongly emphasized by many recently discovered taxa from the Maotianshan Shale, including the oldest ctenophores (5), chaetognaths (18), mollusks (J.-Y.C., D.H., and D. J. Bottjer, unpublished data), sipunculans (D.H., J.-Y.C., J. Vannier, and J. L. Siaz Salinas, unpublished data), and even vertebrates and their stem-lineage (19–22). Here we add the first true tunicate, *Shankouclava*. Although *Shankouclavis* may have been a basal tunicate that lived before the three extant lines of modern tunicates split off (ascidians, thaliacians, and larvaceans), it

resembles some living aplousobranch ascidians (8). Recent phylogenetic evidence from 18S rRNA sequences suggests that *Aplousobranchs* are at or near the base of all tunicates (23). This finding suggests that a basic tunicate body plan, once established, has changed very little over the past 520 million years.

#### Appendix: Systematic Paleontology

##### Subphylum. Urochordata.

##### Genus. *Shankouclava* gen. nov.

**Etymology.** Generic name is a compound of Shankou (fossil locality) and club-shaped (*Latin clava*).

**Diagnosis.** A solitary sessile tunicate (Fig. 3) that is soft-bodied and club-shaped, elongated ( $\approx 2$ –4 cm long) and pointed proximally, with rooted attaching stolons. The body is divided into an anterior part shaped like a barrel and a posterior part shaped like an elongated triangle, with the latter called the “abdomen.” The anterior part, which occupies more than half the length of the body, is dominated by a large pharynx with up to 30 branchial bars. The branchial bars branch to form a rectangular network with stigmata (branchial openings). An oral siphon opens subventrally, containing oral tentacles at its base. An endostyle band extends along the mid-ventral part of the pharynx, and a sac-like atrium surrounds the pharyngeal basket. The alimentary canal is U-shaped, with the anterior gut (esophagus) running posteriorly from the pharynx and forming a loop, called the stomach, near the base of the abdomen. From there, the posterior gut (intestine) extends anteriorly to end near an atrial pore, which lies two-thirds of the way back from the anterior pharyngeal margin. Unique segmentation on the dorsal body. Otherwise, *Shankouclava* rather resembles the individual zooids of some modern, colonial, aplousobranch tunicates.

**Type Species.** *Shankouclava anningense* gen. et sp. nov. (Figs. 1 A–H and 2).

**Etymology.** Specific name refers to Anning county.

**Holotype.** The holotype represents a complete animal, which is compacted laterally and split into part (SK01001a) and counterpart (SK01001b).

**Material.** Eight specimens, four of which are complete.

**Diagnosis.** Same as the generic diagnosis.

**Locality and Stratigraphy.** Shankou Village, Anning County, Kunming; Lower Cambrian Maotianshan Shale *Eoredlichia* Zone.

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1. Brusca, R. C. & Brusca, G. J. (2002) *Invertebrates* (Sinauer, Sunderland, MA), 2nd Ed.
2. Raff, R. A. (1996) *The Shape of Life* (Univ. of Chicago Press, Chicago).
3. Shu, D. G., Chen, L. & Zhang, X. L. (2001) *Nature* **411**, 472–473.
4. Dominguez, P., Jacobson, A. G. & Jefferies, R. P. S. (2002) *Nature* **417**, 841–844.
5. Chen, J. Y. & Zhou, G. Q. (1997) *Bull. Natl. Mus. Nat. Sci.* **10**, 11–105.
6. Chen, J. Y. & Erdtman, B. D. (1991) in *The Early Evolution of Metazoa and the Significance of Problematic Taxa*, eds. Simmoneta, A. M. & Conway Morris, S. (Cambridge Univ. Press, Cambridge, U.K.), pp. 57–75.
7. Chen, J. Y. & Lindström, M. (1991) *Geologiska Föreningens i Stockholm Förhandlingar* **113**, 79–81.
8. Burighe, P. & Cloney, R. A. (1997) in *Microscopic Anatomy of Invertebrate Chordates*, eds. Harison, F. W. & Ruppert, E. E. (Wiley-Liss, New York), Vol. 15, pp. 221–347.
9. Millar, R. H. (1971) *Adv. Mar. Biol.* **9**, 1–100.
10. Barrington, E. J. W. (1965) *The Biology of Hemichordata and Protochordata* (Freeman, San Francisco).
11. Luo, H. L., Hu, S. X., Chen, L. Z., Zhang, S. S. & Tao, Y. H. (1999) *Early Cambrian Chengjiang Fauna from Kunming Region, China* (Yunnan Science & Technology Press, Kunming, China).

12. Jefferies, R. P. S. (1968) *Bull. Br. Mus. Nat. His. (Geology)* **16**, 243–339.
13. Jefferies, R. P. S. (1986) *The Ancestry of the Vertebrates* (British Museum of Natural History, London).
14. Conway Morris, S. (1998) *The Crucible of Creation: The Burgess Shale and the Rise of Animals* (Oxford Univ. Press, Oxford).
15. Briggs, D. E. G., Erwin, D. H. & Coillier, F. J. (1994) *The Fossils of the Burgess Shale* (Smithsonian Institution Press, Washington, DC).
16. Valentine, J. W., Jablonski, D. & Erwin, D. H. (1999) *Development (Cambridge, U.K.)* **126**, 851–859.
17. Chen, J. Y., Oliverli, P., Li, C. W., Gao, F., Hagadom, J. W., Peterson, K. J. & Davidson, E. H. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 4457–4462.
18. Chen, J. Y. & Huang, D. Y. (2002) *Science* **298**, 187.
19. Shu, D. G., Luo, H. L., Conway Morris, S., Zhang, X. L., Hu, X. X., Chen, L., Han, J., Zhu, M., Li, Y. & Chen, L. Z. (1999) *Nature* **402**, 42–46.
20. Chen, J. Y., Huang, D. Y. & Li, C. W. (1999) *Nature* **402**, 518–522.
21. Holland, N. & Chen, J. Y. (2001) *BioEssays* **23**, 142–151.
22. Mallatt, J. & Chen, J. Y. (2003) *J. Morphol.* **256**, 1–32.
23. Stach, J. and Turbeville, J. M. (2002) *Mol. Phylogen. Evol.* **25**, 408–428.