Paired gill slits in a fossil with a calcite skeleton

Patrício Dominguez*†, Antone G. Jacobson‡ & Richard P. S. Jefferies*

* Department of Palaeontology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
‡ Patterson Labs, MCDB, The University of Texas, Austin, Texas 78712, USA
† Departamento de Paleontologia, Universidad Complutense de Madrid, Ciudad Universitaría, 28040 Madrid, Spain

The chordates, hemichordates (such as acorn worms) and echinoderms (such as starfish) comprise the group Deuterostomia, well established as monophyletic[1,2]. Among extant deuterostomes, a skeleton in which each plate has the crystallographic structure of a single crystal of calcite is characteristic of echinoderms and is always associated with radial symmetry and never with gill slits. Among fossils, however, such a skeleton sometimes occurs without radial symmetry. This is true of *Jaekelocarpus oklahomensis*, from the Upper Carboniferous of Oklahoma, USA, which, being externally almost bilaterally symmetrical, is traditionally placed in the group Mitrata (Ordovician to Carboniferous periods, 530–280 million years ago), by contrast with the bizarrely asymmetrical Cornuta (Cambrian to Ordovician periods, 540 to 440 million years ago). Using computer X-ray microtomography, we describe the anatomy of *Jaekelocarpus* in greater detail than formerly possible, reveal evidence of paired gill slits internally and interpret its functional anatomy. On this basis we suggest its phylogenetic position within the deuterostomes.

Three widely divergent views of the fossils known as mitrates and cornutes are now current—one chordate interpretation[3–8], and a first[9–13] and second[14,15] echinoderm interpretation. The present paper redescribes the mitrate *J. oklahomensis* from the Upper Carboniferous Gene Autry Shale Formation near Ardmore, Oklahoma, USA, which was originally described under the second echinoderm interpretation[15]. We redescribe it under the chordate interpretation, initially as a convention so that useful words such as 'ventral' or 'posterior' shall be unambiguous.

Jaekelocarpus, like all other mitrates and also like an extant tunicate tadpole, consists of a head and a tail. We reconstruct its external appearance in Figs 1 and 2.

The tail can be only tentatively reconstructed in *Jaekelocarpus* but, as in other mitrates, is made up of fore-, mid- and hind-tail. It probably served to pull the animal rearwards in life[6,7,13] by the use of big muscles located in the fore tail.

The head was dorsally flat and ventrally convex and its skeleton was constructed of ten calcite plates only. These are given letters intended to imply homology with particular plates in other mitrates and cornutes[4] (Fig. 1). The ventral skeleton would have been rigid and consists of plate θ on the left, ε on the right and π anteriorly. The externally visible dorsal skeleton consists of plate h at posterior right, i at posterior left, d at median anterior and c (oral spine) articulated to the anterior end of d. Also in the dorsal skeleton but inside the animal are plate e in contact with the right margin of d, plate k/l in contact with the left margin of d and plate μ joined to the ventral ends of e and k/l. Plates e, k/l and μ, together with the externally visible d, form a mouth frame not previously described. The mouth was located in a gap between d above and π below. There are two large antero-dorsal gaps in the skeleton, not previously observed. The left antero-dorsal gap was bordered by ventral plate θ laterally and by k/l and d medially while the right antero-dorsal gap was bordered by ventral plate ε laterally and plates d and e medially. These gaps are certainly not due to post-mortem damage because the plates in contact with them are round-edged rather than fractured.

Two complicated structures, here named the left and right slit-bearing complexes, are internal extensions of the ventral plates θ and ε respectively and are rigidly attached postero-dorsally, by a short isthmus of calcite, to the anterior surface of a low vertical wall on the internal surface of those plates. They are built of delicate imperforate stereom about 20 μm or less in thickness. They have, on right and left, a dorsal, almost horizontal imperforate lamina (the 'roof' of the complex) which passes laterally into a ventro-medially sloping lamina (the 'side wall' of the complex) penetrated by at least three slits. These are arranged in a dorso-ventral series (L1, L2 and L3 on the left and R1, R2 and R3 on the right), are horizontally elongate with their dorsal edges almost parallel to the ventral edges and are each connected to a short calcite tube which extends medianwards approximately perpendicularly to the slit and with the same cross-section as the slit. The connection, at the slit, of each
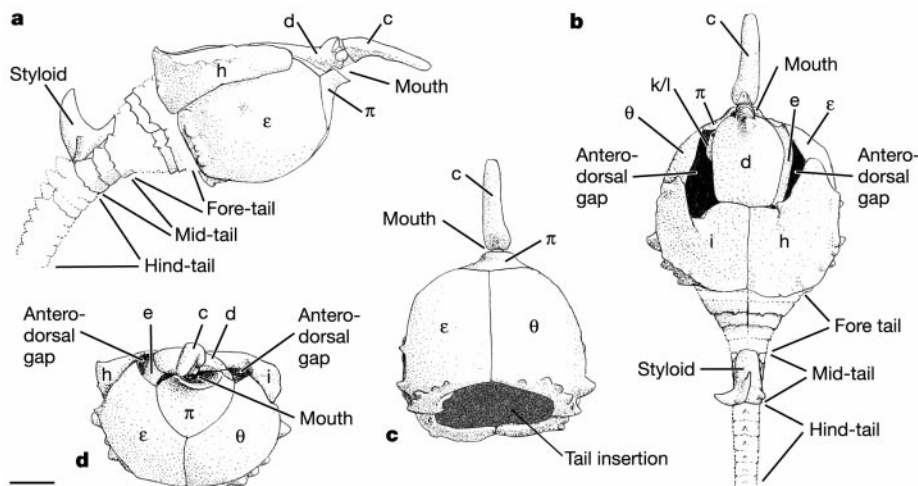
Figure 1 Reconstruction of *Jaekelocarpus oklahomensis* Kolata, Frest & Mapes 1991 (ref. 15) (Gene Autry Shale Formation, Upper Carboniferous, near Ardmore, Oklahoma, USA). The head is reconstructed by X-ray microtomography as shown in Fig. 2, and the tail is reconstructed on the basis of several plates observed in the paratypes. No attempt has been made to reconstruct the soft parts around the mouth nor in the antero-dorsal gaps, but muscles which probably raised and lowered the oral spine c are indicated. For meaning of plate notation, see text. Scale bar, 1 mm. **a**, Right lateral aspect, tail curved downwards as was probably common in life. **b**, Dorsal aspect, tail straight. **c**, Ventral aspect of head. **d**, Anterior aspect of head.
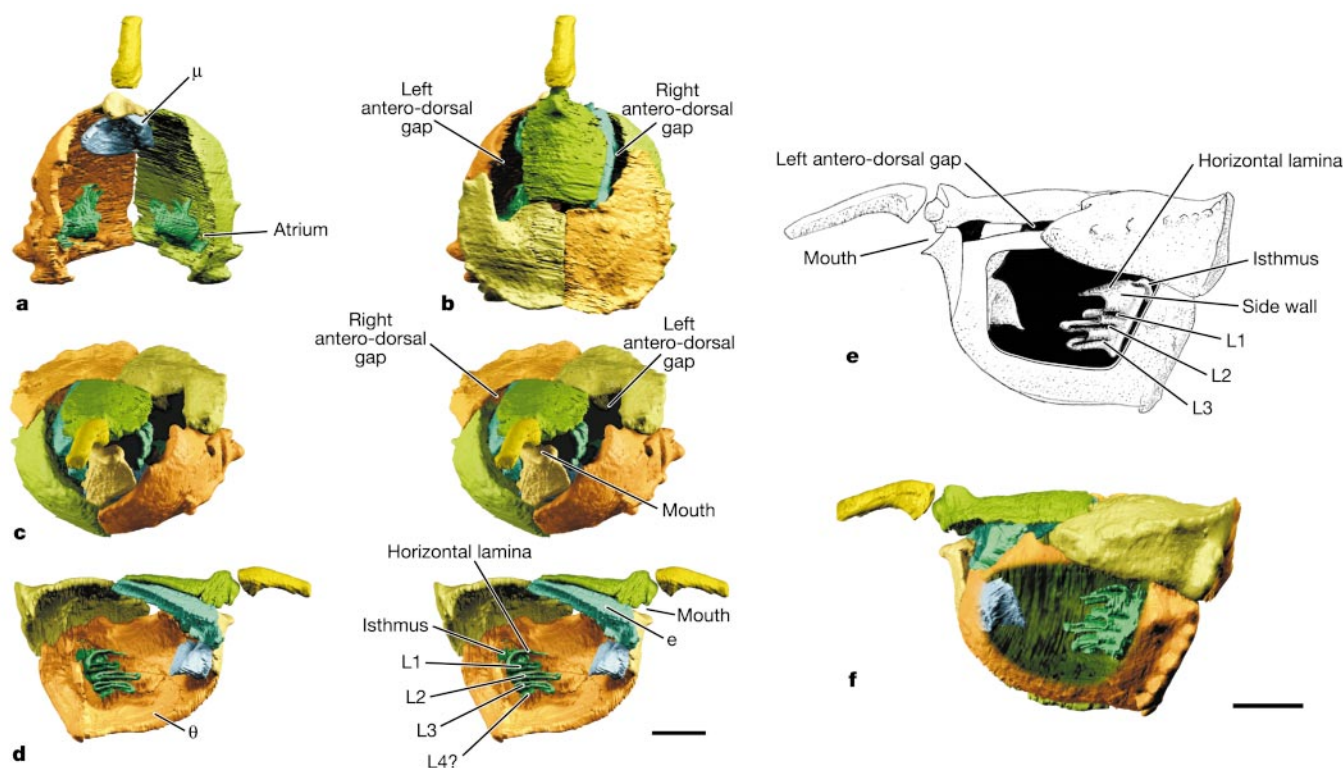
Figure 2 Microtomographic reconstructions of *J. oklahomensis* Burke Museum, University of Washington, UWBM74305. Plates shown in life position. For plate notation see Fig. 1 and text. The applied colouring of a plate distinguishes it from its neighbours, except for the blue-green colour of both slit-bearing complexes, which are parts of plates ε and θ. No attempt has been made to correct damage to the antero-dorsal edge of plate θ or the left and right edges of π. Plates μ, e and k/l are ventral plates of the mouth frame (previously unreported). Panels **c**, **d** are stereo pairs. **a**, Dorsal aspect of ventral skeleton showing internal features including dorsal surfaces of the paired slit-bearing complexes (blue-green). **b**, Dorsal aspect of head. **c**, Antero-dorsal aspect to emphasise the size of the antero-dorsal gaps; π (pale yellow) is incomplete at right and left and ε (orange) is incomplete antero-dorsally. The blue plates belong to the internal mouth frame. **d**, The left slit-bearing complex (blue-green), attached to the rest of plate θ (orange), exposed in median aspect by removal of plates h and ε from the right side of the skeleton; the blue plates belong to the internal mouth frame. **e**, Explanatory drawing of **f**. **f**, Left slit-bearing complex, in lateral aspect, shown in relation to the rest of the skeleton by rendering the rest of plate θ transparent. Scale bars, 1 mm. (See also Supplementary Information.)

tube to the side wall of the slit-bearing complex is not abrupt but is everywhere rounded. In addition to the three pairs of slits already mentioned, there are, on left and right, possible indications of a fourth slit (L4, R4) ventral to the others. There is a clear space lateral to each slit-bearing complex, between it and the internal surface of the rest of plate ε on the right or θ on the left. Because of the rigid isthmus connecting the slit-bearing complex with the rest of the plate that bears it, this space is geometrically required by the skeletal anatomy, not reconstructional. Figure 3 shows the left and right complexes in their post-mortem positions.

The elongate shape of the slits and tubes and their remarkably constant, very low height measured in tens of micrometres (70 to 100 μm; mean 75 μm) which is irrespective of the varying length (range 510–820 μm), strongly suggests that they were lined in life with ciliated epithelia functioning to pump water through the tubes (Table 1). A comparison can be drawn with the pumping ciliated interfilamentary slits in the gills of the bivalve *Mytilus edulis*, the width (height) of which is remarkably constant at about 40 μm (occupied by two opposed sets of 15 μm long cilia together with a

10 μm gap between the two sets), while the length varies but is some two orders of magnitude greater. The width of the slits is known to be physiologically critical in *Mytilus*, because flow through a slit decreases if the gap, normally 10 μm wide, between the two sets of cilia is diminished[16]. On the reasonable assumption that the calcitic slits of *Jaekelocarpus* were lined with epithelia 20 μm thick, say, the dimensional resemblance to the slits of *Mytilus* is remarkable. The main difference is that the slits of *Mytilus* are longer and more numerous, but this is presumably in part because *Mytilus* is much bigger than *Jaekelocarpus*.

The gill slits (stigmata) of ascidian tunicates are similarly ciliated[17]. A provisional statistical study of the size and shape of the stigmata of Australian ascidians based on the scaled, camera-lucida drawings of Kott[18–20] shows that these stigmata range in width (height) from 9 to 60 μm with a mean of about 23 μm (Fig. 4). At least up to a length of about 1 mm, ascidian stigmata have a weak tendency to increase in width as the length increases and the slits of *Jaekelocarpus* are longer than those of most ascidians. In consequence, assuming that the calcitic slits were lined with epithelia
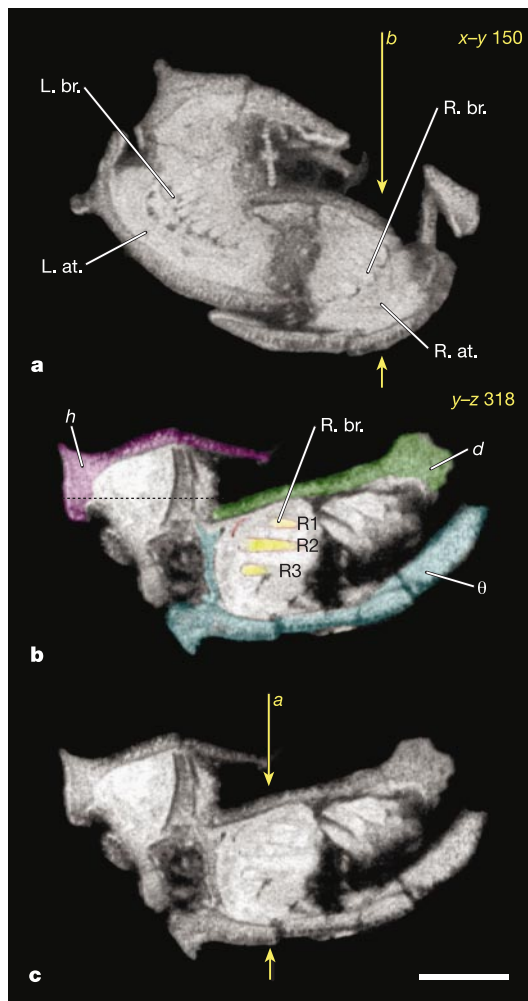
Table 1 Dimensions of the slits in micrometres

Left	Slit length	Mean height*	Tube length	Right	Slit length	Mean height*	Tube length
L1	510	75	450	R1	640	75	520
L2	820	75	380	R2	>380†	75	390
L3	720	75	280	R3	760	75	190

A possible fourth slit has been ignored.
*The range in height for the slits is 70–100 μm.
†Incomplete slit.

Figure 3 Microtomographic sections of *J. oklahomensis* Burke Museum, University of Washington, UWBM74305. **a**, Transverse section (original X-ray tomographic section no. 150, plane *x–y*) of left and right slit-bearing complexes in their post-mortem positions in the plates which bear them (θ to right, ε to left). The yellow arrows show the intersection of **b** and **c** with **a**. **b**, **c**, Secondarily generated longitudinal section (no. 318, plane *y–z*); the yellow arrows in **c** show the intersection of **a** with **b** and **c**. **b**, Projection (yellow) of the median ends of the tubes of slits R1, R2 and R3 onto plane b of **a**. **c**, As **b**, but uncoloured, to show the objective evidence for the position of the branchial slits. Scale bar, 1 mm. L. at., left atrium; L. br., left branchial slit; R. at., right atrium; R. br., right branchian slit.

between 7.5 and 20 μm thick, they would fall naturally along the regression line calculated for the ascidians (Fig. 5).

As already mentioned, each slit has a short calcitic tube projecting inwards from it. Water probably flowed outwards, first through the tube and then through the slit. This is suggested by comparison with
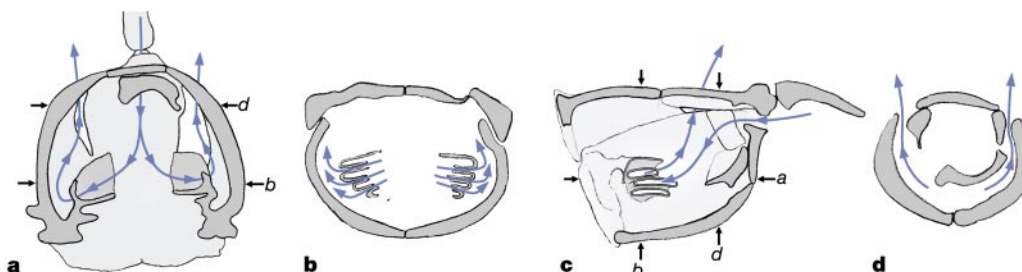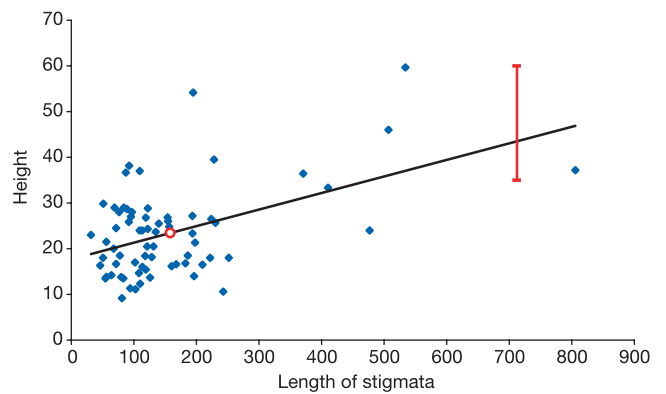
Figure 5 Length versus height (in μm), regression line (black) and mean (red circle) of ascidian stigmata (blue diamonds) in relation to the slits of *Jaekelocarpus* (vertical red line), assuming that the calcitic slits of the latter (mean height 75 μm) were lined with an epithelium between 7.5 and 20 μm thick, giving an estimated height between 35 and 60 μm. The data points (blue diamonds) for extant ascidian stigmata were taken from the scales drawings of Kott[18–20]. Each point represents the mean value for a species (69 species; *n* = 421 stigmata).

the gill slits of cornutes (always on the left side of the head only) which are presumably broadly homologous with the left gill slits of mitrates and which often have a plausible outlet-valve structure[3,21,22].

After flowing outwards through a slit (Fig. 4) water probably entered an unpaired chamber (atrium), or a pair of chambers (atria), which would have filled the space visible in transverse sections lateral to the slit-bearing complexes but internal to the rest of plates ε and θ. This chamber or chambers probably debouched through the large antero-dorsal gaps left and right of plate d, which would otherwise have no obvious function.

The slits of *Jaekelocarpus* have been suggested to be respiratory[15], and perhaps gill slits[21]. These suggestions were made, however, on the basis of the broken material studied and drawn by Kolata *et al.*[15]. X-ray microtomography strengthens the comparison with gill slits by revealing the anterior boundaries of the slits for the first time and yielding dimensions consistent with the presence of a ciliary pump. This functional analysis suggests two general conclusions:

(1) The slits in the slit-bearing complexes of *Jaekelocarpus* were probably ciliated water-pumping gill slits, passing water outwards. Water passing out from the slits would enter the spaces (atria) lateral to the slits and, probably, would escape from the animal through the antero-dorsal gaps described here for the first time. Among extant chordates, such ciliated slits occur in acraniates (for example, amphioxus = *Branchiostoma*) and tunicates.

(2) *Jaekelocarpus* was probably a tunicate because it shows several potential synapomorphies with extant tunicates. These are that the probably ciliated gill slits were antero-posteriorly elongate like the

Figure 4 Suggested flow of water through the head of *Jaekelocarpus*. **a**, Ventral aspect. **b**, Transverse section through the slits. **c**, Sagittal section and left half of head. **d**, Transverse section through oral frame. Water (arrows show flow) enters through the mouth, passes through the slits at right and left (see **a**, **b**, **c**) into the left and right atria (see **b**, **c**) and leaves the head through the antero-dorsal gaps (see **a**, **c**, **d**).

 843

stigmata of ascidian tunicates[23], arranged in paired dorso-ventral columns like the stigmata of ascidian and doliolid tunicates[23], while the presumed atria extended far forwards right and left of the stigmata and therefore right and left of the pharynx, as in ascidian tunicates[23]. Furthermore, the atria opened antero-dorsally as in ascidian tunicates[23], and these anterior openings were paired as in post-larval ascidian tunicates[24].

As a tunicate, *Jaekelocarpus* probably belonged to the stem group of the Tunicata because it retained the primitive features of a calcite skeleton and a downward-flexing tail which, as parsimony suggests, would not have existed in the latest common ancestor of extant tunicates. If a tunicate, *Jaekelocarpus* would necessarily belong to the crown group of the chordates, as the chordate interpretation of mitrates and cornutes has long proposed for mitrates in general[22,25]. □

Methods

The technique we used is high-resolution X-ray computed tomography (μCT). It is non-destructive and permits the study of non-transparent objects such as fossils, producing images that correspond to serial sections with resolution as good as 8 μm. The sections were used to create three-dimensional computer models of the objects by reconstructing the surfaces that connected corresponding outlines on adjacent sections[26,27].

The specimen of *Jaekelocarpus oklahomensis* chosen for complete scanning (Burke Museum, University of Washington, UWBM74305) was selected after preliminary tests on a total of three specimens. The scanning was carried out, under the direction of T. Rowe, at the University of Texas High-Resolution X-Ray CT Facility, Department of Geological Sciences, USA. The parameters of the scan were: l50 kV; 0.053 mA; slice thickness 0.016 mm; interslice spacing 0.016 mm; diameter of field of view 4.53 mm. 276 sections were saved as 16-bit TIFF files. The matrix data size was 512 × 512 × 376 voxels and the voxel size was 8.84 × 8.84 × 16 μm (see Supplementary Information).

The individual plates were recognized, and distinguished from each other as separate entities (segmented), using Mimics 6.3 software (Materialise N. V.). In Mimics, the data can be explored in three views: the original images in *x–y* planes and resliced images in *x–z* or *y–z* planes. Because of lateral changes in the X-ray attenuation values within individual skeletal plates, it was necessary to use local thresholds.

The CTM software module of Mimics interpolates the slice data to generate three-dimensional STL files, one for each plate or anatomical region. Such STL files were exported to Rhinoceros 1 (Robert McNeel) as polygon meshes, where models of the plates were moved in virtual space and so placed in the original life positions relative to each other. To prevent artefacts after these manipulations, the size parameters of the plates (length and volume) were checked in Communicator 1.6 (Materialise N. V.) by means of which we obtained secondary serial sections of the restored specimen.

1. Peterson, K. J. & Eernisse, D. J. Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S r DNA. *Evol. Dev.* **3,** 170–205 (2001).
2. Cameron, C. B., Garey, J. R. & Swalla, B. J. Evolution of the chordate body plan: new insights from phylogenetic analyses of deuterostome phyla. *Proc. Natl Acad. Sci. USA* **97,** 4469–4474 (2000).
3. Jefferies, R. P. S. Some fossil chordates with echinoderm affinities. *Symp. Zool. Soc. Lond.* **20,** 163–208 (1967).
4. Jefferies, R. P. S. in *Major Events in Vertebrate Evolution* Ch. 4 (ed. Ahlberg, P. E.) 20–66 (Systematics Association and Taylor & Francis, London, 2000).
5. Jefferies, R. P. S. & Jacobson, A. G. An episode in the ancestry of the vertebrates: from mitrate to crown-group craniate. *Integr. Biol.* **1,** 115–132 (1998).
6. Sutcliffe, O. E., Südkamp, W. H. & Jefferies, R. P. S. Ichnological evidence on the behaviour of mitrates: two trails associated with the Devonian mitrate: *Rhenocystis. Lethaia* **33,** 1–12 (2000).
7. Jefferies, R. P. S. Ichnological evidence on the behaviour of mitrates: comments on the reply. *Lethaia* **34,** 262 (2001).
8. Cripps, A. P. A new stem-group chordate (Cornuta) from the Llandeilo of Czechoslovakia and the cornute-mitrate transition. *Zool. J. Linn. Soc.* **96,** 49–85 (1989).
9. Ubaghs, G. in *Treatise on Invertebrate Paleontology Part S. Echinodermata* (ed. Moore, R. C.) S297–S650 (Kansas Univ. Press and Geological Society of America, New York, 1968).
10. Parsley, R. L. in *Echinoderm Phylogeny and Evolutionary Biology* (eds Paul, C. R. C. & Smith, A. B.) 347–361 (Oxford Scientific Publications/Liverpool Geological Society, Oxford/Liverpool, 1988).
11. Ruta, M. Brief review of the stylophoran debate. *Evol. Dev.* **1,** 123–135 (1999).
12. David, B., Lefebvre, B., Mooi, R. & Parsley, R. L. Are homalozoans echinoderms? An answer from the extraxial-axial theory. *Paleobiology* **26,** 529–555 (2000).
13. Lefebvre, B. & David, B. Ichnological evidence on the behaviour of mitrates: reply. *Lethaia* **34,** 260–261 (2001).
14. Philip, G. M. Carpoids: echinoderms or chordates? *Biol. Rev.* **54,** 439–471 (1979).
15. Kolata, D. R., Frest, T. J. & Mapes, R. H. The youngest carpoid: occurrence, affinities and life mode of a Pennsylvanian (Morrowan) mitrate from Oklahoma. *J. Paleontol.* **65,** 844–855 (1991).
16. Jørgensen, C. B. Water processing in ciliary feeders, with special reference to the bivalve filter pump. *Comp. Biochem. Physiol.* **94a,** 383–394 (1989).
17. Martinucci, G. B., Burighel, P. & Dallai, R. in *Form and Function in Zoology* (eds Lanzavecchia, G. & Valvassori, R.) **Vol. 5,** 123–140 (Selected Symposia and Monographs, Unione Zoologica Italiana, Mucchi, Modena, 1991).
18. Kott, P. The Australian Ascidiacea. Part 1. Phlebobranchia and Stolidobranchia. *Memoirs Queensland Mus.* **20,** 1–47 (1985).
19. Kott, P. The Australian Ascidiacea. Part 2. Aplousobranchia. *Memoirs Queensland Mus.* **29,** 1–298 (1990).
20. Kott, P. The Australian Ascidiacea. *Memoirs Queensland Mus.* **32,** 375–620 (1992).
21. Jefferies, R. P. S. A defence of the calcichordates. *Lethaia* **30,** 1–10 (1997).
22. Jefferies, R. P. S. *The Ancestry of the Vertebrates* (Cambridge Univ. Press and British Natural History Museum, London, 1986).
23. Berrill, N. J. *The Tunicata with an Account of the British Species* (Ray Society, London, 1950).
24. Huus, J. Genitalorgane und Ganglio-Genital-Strang bei *Corella parallelogramma* O. F. M. *Skrifter. VidenskabSelskabet Christiania.* **19,** 1–50 (1924).
25. Jefferies, R. P. S. In defence of the calcichordates. *Zool. J. Linn. Soc.* **73,** 351–396 (1981).
26. Ketcham, R. A. & Carlson, W. D. Acquisition, optimization and interpretation of X-ray computed tomographic imagery; applications to the geosciences, three-dimensional reconstruction, modeling and visualization of geological materials. *Comp. Geosci.* **27,** 381–400 (2001).
27. van Geet, Swennen, R. & Wevers, M. Quantitative analysis of reservoir rocks by microfocus X-ray computerised tomography. *Sedim. Geol.* **132,** 1–2 (2000).

··

Positive interactions among alpine plants increase with stress

Ragan M. Callaway*, R. W. Brooker†, Philippe Choler‡, Zaal Kikvidze§, Christopher J. Lortie‖, Richard Michalet¶, Leonardo Paolini#, Francisco I. Pugnaire☆, Beth Newingham*, Erik T. Aschehoug*,**, Cristina Armas☆, David Kikodze§ & Bradley J. Cook*

* *Division of Biological Sciences, University of Montana, Missoula, Montana 59812, USA*
† *Centre for Ecology and Hydrology, CEH Banchory Research Station, Hill of Brathens, Banchory, AB31 4BY, UK*
‡ *Station Alpine du Lautaret et Laboratoire de Biologie des Populations d'Altitude UMR CNRS-UJF 5553, University Joseph Fourier, Grenoble, BP 53, 38041 Grenoble cedex, France*
§ *Institute of Botany of the Georgian Academy of Sciences, Kojori Road 1, Tbilisi 380007, Georgia*
‖ *Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada*
¶ *Ecosystemes et Changements Environnementaux, University Joseph Fourier, Grenoble, BP 53, 38041 Grenoble cedex, France*
# *Laboratorio de Investigaciones Ecológicas de las Yungas, UNT, CC34, Yerba Buena, Tucuman, Argentina*
☆ *Estacion Experimental de Zonas Aridas, Consejo Superior de Investigaciones Cientificas, General Segura 1, 04001 Almeria, Spain*

....

Plants can have positive effects on each other[1]. For example, the accumulation of nutrients, provision of shade, amelioration of disturbance, or protection from herbivores by some species can enhance the performance of neighbouring species. Thus the notion that the distributions and abundances of plant species are independent of other species may be inadequate as a theoretical underpinning for understanding species coexistence and

** *Present address: The Nature Conservancy, 201 Mission Street, San Francisco, California 94105, USA.*