upper-mantle carbon supplies. The first possibility is difficult to quantify, but can be visualized conceptually. During the early stages of mantle melting the ferric–ferrous ratios of the melt and residual solid will be determined by values of f_{O_2} at or close to CCO. If carbon is consumed during melting the ferric–ferrous ratio of the melt will decrease unchecked until the melt segregates from its source. The extent to which carbon-absent melting will proceed is unknown, although the large variation of f_{O_2} in MORB glasses (Fig. 1*b*) may reflect this process as well as the aggregation of polybaric mantle melts. The second possibility is addressed by Wood *et al.*[3] with reference to the source of island-arc basalts. The third possibility may also be apropriate to subduction zones where appreciable H_2O is introduced into the mantle by the subducting slab[31]. If the $H_2O/(H_2O+CO_2)$ ratio in the hybrid mantle fluids exceeds ~0.9, any mantle carbon will be completely oxidized by H_2O (ref. 26), enabling f_{O_2} to rise unbuffered above CCO. We propose that recycling of hydrous fluids in subduction zones may account for the relatively oxidizing nature of subduction-related peridotites[3,4] (Fig. 1*a*) and the more oxidized nature of arc magmas in general[32].

Carbonate-bearing equilibria (2) and (3) may control f_{O_2} in the deeper parts of the mantle[5], for example, at pressures >12 kbar along the geotherm of the southwestern United States (Fig. 1*a*). Although carbonate is rare in mantle xenoliths, including those in Fig. 1*a*, it has recently been shown experimentally[33] that carbonates in mantle assemblages rapidly decompose on decompression. Thus the apparent lack of carbonate in mantle samples in the quantities required to control f_{O_2} (~0.05 vol% $MgCO_3$) may be entirely an artefact of the volcanic processes by which they are brought to the surface. ☐

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Owenetta and the origin of turtles

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THE origin and relationships of turtles have fascinated and puzzled generations of palaeontologists. Among living amniotes only turtles, crocodiles and mammals have substantial fossil records, extending into the Triassic (200 Myr). These vertebrates have attracted much attention and the broader aspects of crocodilian and mammalian evolutionary relationships are relatively well known. Therefore, it is surprising that the origins and relationships of the Testudines have remained unresolved. Numerous groups of extinct tetrapods[1–7] have been cited as possible turtle relatives, including the Captorhinidae[8–12]. New specimens of the small reptile *Owenetta* from the Upper Permian and Lower Triassic sediments of South Africa provide strong evidence that a group of primitive amniotes, the procolophonids, are the closest sister-group of turtles.

Procolophonid reptiles are small to medium-sized primitive amniotes that first appear in late Palaeozoic (Upper Permian) deposits of South Africa. The fossil record of procolophonids extends to the end of the Triassic (early Mesozoic), and indicates that this was a diverse and successful group with worldwide distribution. Procolophonids are the last known members of the initial adaptive radiation of amniotes, and were probably ecological analogues to some of the small and medium-sized diapsid reptiles that eventually replaced them. Procolophonids are characterized by the following osteological features: the orbital shelf of the prefrontal has a medial process, the ventral edge of the skull is concave in the region of the jugal and the quadratojugal, the ectopterygoid is reduced in size and lacks teeth, and despite their relatively small size, procolophonids have three sacral vertebrae. The early procolophonids have simple peg-like teeth, whereas the advanced Triassic forms develop bulbous, transversely expanded teeth. The larger Triassic procolophonids develop posterolateral horn-like projections on the quadratojugal.

The oldest known procolophonid is *Owenetta*[13,14], a small insectivorous reptile recovered from Upper Permian sediments of the Karroo, South Africa. The study of recently discovered skulls and articulated skeletons (Fig. 1) of this poorly known, primitive procolophonid has led to the discovery of numerous shared derived osteological features (synapomorphies) between procolophonids and turtles. Comparisons between procolophonids such as *Owenetta* and turtles have also been facilitated by a recent thorough redescription of the oldest known turtle, *Proganochelys*[12] (Fig. 2), and the discovery of the Early Jurassic turtle, *Kayentachelys*[15]. Detailed new information about the cranial anatomy of these forms is particularly important because the great specialization of the postcranial skeleton of all turtles (associated with the development of the shell) appears to have obscured most evidence (synapomorphies) of testudine origins and relationships.

Procolophonids and testudines form a monophyletic group that excludes all other amniotes because they share the following derived features which appear in the primitive condition in all other Palaeozoic reptiles: (1) the cultriform process is greatly reduced in length; (2) the teeth on the transverse flange of the pterygoid are lost, and are replaced by a ventral ridge; (3) a distinctly shaped anterodorsal expansion of the maxilla is formed directly posterior to the external naris; (4) the prefrontal and palatine are massively buttressed against each other; (5) the dorsal process of the quadrate is exposed laterally, but the

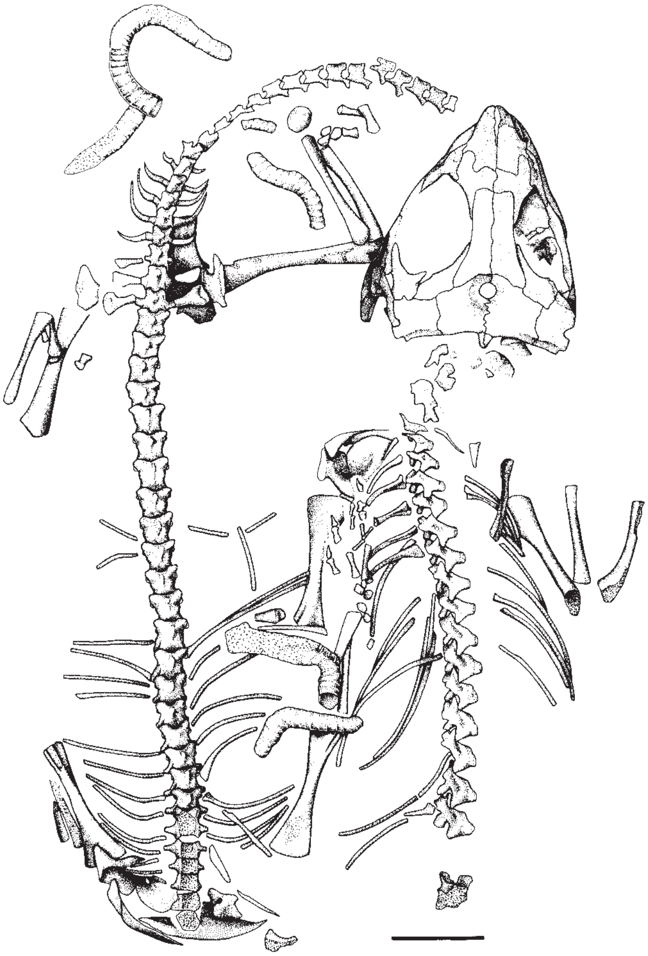

FIG. 1 Two articulated skeletons of the primitive procolophonid *Owenetta* from Tweefontein, Bethulie, South Africa (Cistecephalus zone, Beaufort Series, Lower Triassic). The specimen (BPI 4195) is housed at the Bernard Price Institute of Paleontology, University of Witwatersland, Johannesburgh, South Africa. The neck and head of one skeleton and the pelvis and tail of the other were destroyed by surface erosion before the discovery of this specimen. Scale bar, 1 cm. Small, millepede-like arthropods have been found in abundance in the rib cage and near the skeletons, indicating that these small scavenging arthropods were probably feeding on the two carcasses before burial and fossilization. *Owenetta* has been included in the Family Procolophonidae by all previous palaeontologists who studied this poorly known reptile, including Gow[14], who describes some of the features that we discuss, but did not realize that they were synapomorphies with testudines. We do not include the enigmatic nyctyphruretids from the late Permian of the USSR within the Procolophonidae.

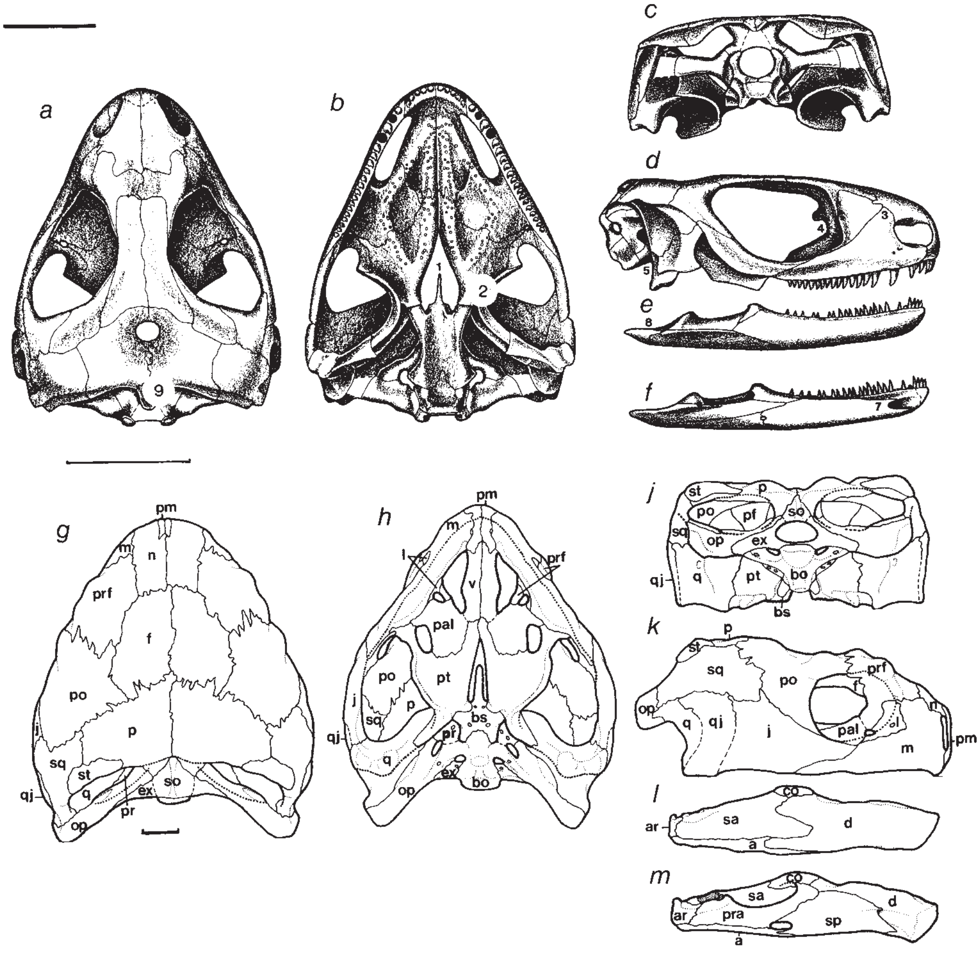

FIG. 2 Reconstructions of *Owenetta* (*a–f*) and *Proganochelys* (*g–m*) skulls in dorsal (*a*, *g*), ventral (*b*, *h*), occipital (*c*, *j*), and lateral (*d*, *k*) views, and mandible in lateral (*e*, *l*) and medial (*f*, *m*) views. *Owenetta*, originally described on the basis of a partial skull from New Bethesda, Graaff Reinet, South Africa (Daptocephalus zone, Beaufort Series, Upper Permian), is now represented by numerous skulls from the same region. The reconstructions are based on specimen shown in Fig. 1. *Proganochelys* is the oldest known turtle, from the late Triassic of Germany. The outline skull reconstructions of *Progranochelys* are modified slightly from that of Gaffney[12]. Abbreviations: a, angular; ar, articular; bo, basioccipital; bs, basisphenoid; co, coronoid; d, dentary; ex, exoccipital; f, frontal; jugal; l, lacrimal; m, maxilla; n, nasal; op, opisthotic; p, parietal; pal, palatine; pf, postfrontal; pm, premaxilla; po, postorbital; pr, prootic; pra, prearticular; prf, prefrontal; pt, pterygoid; q, quadrate; qj, quadratojugal; sa, surangular; so, supraoccipital; sp, splenial; sq, squamosal; st, supratemporal; v, vomer. The numbers refer to the synapomorphies discussed in the text.

edge of the well developed tympanic notch is formed by the squamosal and the enlarged quadratojugal (also present in *Proganochelys* (personal observation), although not possible to show in Fig. 2*k*); (6) the slender stapes has lost both its dorsal process and foramen (not shown in the figures); (7) on the lower jaw the distinctly shaped anterior edge of the splenial is excluded from the symphysis; (8) the dorsal surface of the retroarticular process is broad and concave, and is formed by at least three bones, the articular, angular and prearticular; (9) the postparietal is greatly reduced or lost (see Fig. 2 for illustration of these points); (10) the entepicondylar foramen of the humerus is lost. No other group of Palaeozoic reptiles has these derived features. Although some Palaeozoic reptiles[16] appear to possess a tympanic notch, the configuration of the surrounding elements is entirely different from those of procolophonids and turtles. Advanced diapsids also reduce and eliminate the postparietal, but the most primitive diapsids retain the primitive condition of well developed postparietals[17]. Even though only one postcranial synapomorphy (the loss of the entepicondylar foramen) can be recognized, this derived condition is not seen in any other Palaeozoic reptile.

Such a strong case has never been made to demonstrate close relationships between turtles and any other group of primitive tetrapods. By contrast, the most recent widely accepted hypothesis of close captorhinid–turtle relationships[10,12] is supported by only four synapomorphies. Two of these characters, however, the absence of the tabular and the presence of the foramen orbito-nasale, are not unique to turtles and captorhinids, but are also found in other reptiles, including procolophonids.

In addition to solving a problem that has puzzled generations of palaeontologists, our results could alter considerably our concepts of turtle evolution. The use of procolophonids (instead of captorhinids) as the outgroup of turtles requires changes to the diagnosis of the Testudines: both the presence of a solid, rod-like imperforate stapes and the distinctive morphology of the quadrate–squamosal–quadratojugal complex (probably associated with the development of a tympanum and an impedance-matching middle ear) have been used to diagnose turtles[10], but are also present in procolophonids. These new results also alter significantly our interpretation of the testudine fossil record. If captorhinids are the closest sister-group of turtles, then the Testudines must have arisen at least as early as the early Permian, and possibly the late Carboniferous. As the oldest known turtle comes from Upper Triassic strata, this implies an unusually long gap in the fossil record (more than 100 Myr), longer than any other group of modern reptiles. If, however, Testudines is the sister-group of procolophonids, then this group of extant reptiles may have originated as late as the late Permian. This second hypothesis implies a much shorter gap and hence the fossil record associated with the origin of turtles may not be as incomplete as previously thought. □

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Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum

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CONJUNCTIVE stimulation of climbing and parallel fibres in the cerebellum evokes a long-term depression of parallel-fibre Purkinje-cell transmission[1,2], a phenomenon implicated as the cellular mechanism for cerebellar motor learning[3]. It is suspected that the increase in cyclic GMP concentration that occurs after activation of climbing fibres[4] is required to evoke long-term depression[3]. Excitatory amino acids are known to cause the release of nitric oxide (NO), resulting in elevation of the cGMP level in the cerebellum[5]. Here we report that endogenous NO is released after stimulation of climbing fibres, that long-term depression evoked by conjunctive stimulation of parallel and climbing fibres is blocked by haemoglobin (which strongly binds NO) or L-N^G-monomethyl-arginine (an inhibitor of NO synthase), and that exogenous NO or cGMP can substitute for the stimulation of climbing fibres to cause long-term depression in rat cerebellar slices. These results demonstrate that the release of endogenous NO is essential for the induction of synaptic plasticity in the cerebellum.

Release of NO was recorded by electrochemical NO probes[6] in the molecular layer of cerebellar slices. Stimulation of white matter (20 Hz, 5 s) evoked increases in NO concentration of between 20 and 75 (47 ± 3, ± s.e.m., $n = 20$) nM. The endogenous NO response showed a similar dependence on the cathode voltage of the NO probe as did probe currents induced by a standard NO solution (100 nM; Fig. 1*a*, *b*). Haemoglobin (bovine, 10 μM), which strongly binds NO (refs 5,6), attenuated the NO response by 46–58% ($n = 3$; Fig. 1*c*). L-N^G-monomethyl arginine (NMMA, 100 μM), an inhibitor of NO synthase (ref. 7), blocked the NO response by 82–87% ($n = 3$). This effect was antagonized by L-arginine (Arg, 1 mM; Fig. 1*d*).

Release of NO was also recorded in slices obtained from rats treated with 3-acetylpyridine (3AP), which selectively produces lesions in the inferior olive and removes climbing fibres[8] (Fig. 1*e*). The estimated NO release was between 5 and 13 (9 ± 2, $n = 5$) nM, and was significantly less than that in normal slices ($P < 0.001$), Mann-Whitney U-test). A low-Ca^{2+} (0.24 mM) medium blocked the NO release almost completely and reversibly in normal slices (Fig. 1*f*). Among various glutamate blockers, L-glutamic acid diethyl ester (GDEE, 10 mM) suppressed the NO response by 61–66% ($n = 3$; Fig. 1*g*), while kynurenate (10 mM), 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX, 10 μM) and D-2-amino-5-phosphonovaleric acid (APV, 100 μM) showed no clear effect ($n = 3$ for each). These results suggest that release of endogenous NO is triggered by a transmitter released from climbing fibres, which may activate unknown receptors.

Long-term depression can be monitored as changes in the extracellular potassium concentration ($[K^+]_O$) after stimulation of parallel fibres in the molecular layer[9]. Conjunctive stimulation of parallel fibres and the white matter evoked long-term depression of the K^+ response (Fig. 2*a*, *b*). Depression was also tested in slices treated with 3AP (Fig. 2*c*), and found to be