

FIG. 4 Cladogram showing placement of *Vorona* within Aves. The preserved character states of *Vorona* were scored into the data matrix (Appendix 2) of ref. 21 with the following modifications: (1) characters 36, 59, 63 and 69 were transformed into multistate characters, (2) *Concornis* was included within Enantiornithes²¹, (3) *Mononykus* was added to the ingroup taxa, (4) characters 72, 73, 74, 75, 77, 78, 80, 81 and 84 were eliminated as they occur only in Enantiornithes, (5) on the basis of new evidence from recently prepared specimens, character 79 was rescored for *Patagopteryx* from 0 to 1. Two characters were appended to this data set: character 3 (Appendix 3 of ref. 21) and one new character (narrow, deep intercondylar sulcus on tibiotarsus). The resulting 77 character data set was processed using the implicit enumeration option (Hennig 86; steps = 106, consistency index = 0.77, retention index = 0.83). Given this limited data set (both in taxa and characters), the unambiguous synapomorphies diagnosing the nodes are: NODE 1: prominent ventral processes on cervico-dorsal vertebrae, ossified sternal keel, carpometacarpus, fused pelvic elements, prominent antitrochanter, pubic foot absent, trochanteric crest, caudal trochanter absent, fibula greatly reduced; NODE 2: pygostyle, strut-like coracoid, scapula with sharp caudal end, humerus shorter than ulna, shaft of radius thinner than ulna; NODE 3: synsacrum formed by more than 8 vertebrae, heterocoelus cervical vertebrae, scapulocoracoid articulation well below shoulder end of coracoid, humerus with well developed transverse ligamental groove, distal tarsals completely fused to metatarsals; NODE 4: sagittally curved scapula, extensor canal on tibiotarsus; NODE 5: quadrate orbital process pointed, articular pneumatized, fewer than 11 dorsal vertebrae, ossified uncinatous processes, procoracoid process, humeral head globous, small acetabulum, pubis parallel to ilium, pubic shaft laterally compressed, prominent patellar groove, cranial cnemial crest, tubercle for *m. iliofibularis* caudolaterally or caudally directed, proximal metatarsal III plantarly displaced, well-developed tarsometatarsal intercondylar eminence.

the diversity of early birds¹⁶. Previously, the earliest established record of Malagasy birds was from the early Holocene epoch¹⁷. *Vorona* bears no close phylogenetic relationship to the extant and recently extinct malagasy avifauna, all of which belong to modern higher groups (Neornithes). □

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Nautilus and the art of metabolic maintenance

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THE cephalopod mollusc *Nautilus* is often called a living fossil because its multichambered shell resembles that of extinct forms from the early Palaeozoic era. Living at depths of 100–300 m, the animals not only encounter zones of low oxygen concentration, but also exploit them for refuge or feeding^{1,2}. Despite some modest recruitment of anaerobic sources of energy, *Nautilus* is able to survive severe bouts of hypoxia, mainly through its prodigious capacity for aerobic metabolic rate suppression. Here we show that the hypometabolic, hypoxic animal conserves energy further by means of prolonged ventilatory and circulatory pauses, during which time the blood, having a comparatively high oxygen affinity, is apparently loaded at the venous side, across the superficially located and voluminous vena cava. Under these highly arrested conditions, a significant fraction of the animal's aerobic metabolic rate can be accounted for by a slow 'metering out' of the O₂ store contained in the shell, indicating that the buoyancy chambers of *Nautilus* may occasionally subserve the role of a 'SCUBA tank'. The internal shell morphology and siphuncular arrangement seen in the fossilized remains of ammonites suggest that similar processes may have occurred.

Nautilus pompilius L. living at depths of 225–300 m were taken in baited traps from the sunken barrier reef south-east of Port Moresby, Papua New Guinea, and transported to the Motupore Island research station where they were acclimated to experimental temperatures (18 °C) which approximate those of their 'vertic niche'¹. Unlike other cephalopod molluscs, *Nautilus* is very tolerant to hypoxia². In the short term, this might be expected of any mollusc that bases its defensive strategy on withdrawal into a shell (to out-wait a persistent predator). But *Nautilus* can also remain active for long periods of time as it travels through waters of decreasing oxygen content. It does so by progressively depressing its metabolic rate to match the amount of ambient O₂ available (Fig. 1). *Nautilus* can also survive for at least a day in the most severe hypoxic environments that are ever likely to be encountered naturally², by suppressing its aerobic metabolic rate to 4–8% of the level seen at normal oxygen concentrations (Fig. 1). These rates are equivalent to the hypometabolic feats of well-known 'facultative anaerobes'^{3,4}. This represents a true metabolic suppression (that is, a decrease in heat production), because the estimates of ATP turnover in severe hypoxia, calculated from the aerobic metabolic rate and the accumulation in muscle of

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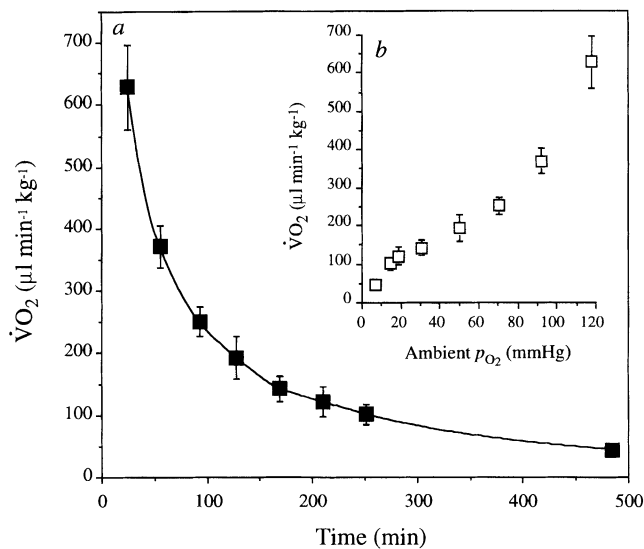


FIG. 1 a, Oxygen consumption ($\dot{V}O_2$) during progressive hypoxia. b, Oxyconformation response to lowered ambient partial pressures of oxygen (p_{O_2}). Oxygen uptake was measured at 18 °C in a thermostatted, gas-tight, 5-litre perspex respirometer, using an EIL 7130 oxygen electrode and Kent O_2 meter. Circulation within the tank was maintained by a magnetic stirrer. p_{O_2} of the closed water system rose by 2–3 mmHg during the course of the experiments. Values are means (error bars s.e.m.) of experiments on 6 animals whose body weights were between 450 and 650 g, corresponding to flesh weights of 300–450 g.

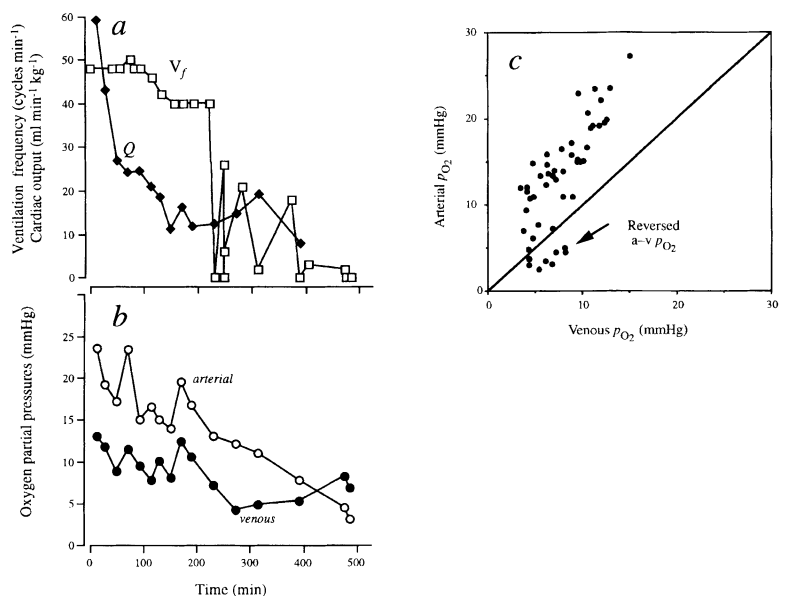
anaerobic metabolic end-products (Table 1), indicate that the recruitment of anaerobiosis makes up for less than 5% of the energy deficit arising from the lack of oxygen.

With such vast reductions in metabolic rate comes a decrease in activity, with brief periods of jet propulsion punctuating longer periods at rest². During these inactive periods, ventilatory movements may cease altogether, cardiac output is dramatically reduced (Fig. 2), and the heartbeat can slow to one or two cycles per min of very low amplitude². As the oxygen partial pressure (p_{O_2}) of arterial blood decreases eventually to 5 mmHg and beyond, the animals remain quiescent for longer and longer periods between bouts of activity. At these times, a most unusual,

but nevertheless repeatable, observation is the occasional reversal of the normal arterial–venous blood p_{O_2} gradient, when venous p_{O_2} actually exceeds that of the arterial blood (Fig. 2a, b). Calculation of the corresponding cardiac output by the Fick equation at this time obviously gives the embarrassing result that blood is flowing in reverse. We are reasonably certain this is not the case, and instead we believe that blood flow is so slow and intermittent during severe hypoxia that the vena cava, which is positioned superficially in the roof of the mantle cavity and is of considerable volume, has time to equilibrate with the ambient p_{O_2} . At the same time, the arterial cannula in the heart picks up blood that has been stationary in the ventricle for some time (although there is still a heart rate, the times between beats can exceed the times during which blood samples might be taken). When ventilatory movements have all but ceased, it therefore seems that the vena cava becomes in effect a functional gill, scavenging what little O_2 remains available, in a most energy-efficient manner.

The extent to which *Nautilus* can exploit the oxygen stores in the blood as well as in the buoyancy chambers of the shell is disputed^{2,5–7}. The O_2 store in the shell chambers of an animal resting at 18 °C are estimated at 6.9 ml for a 450-g (flesh weight) *Nautilus*, with an available shell-gas volume of 100 ml (ref. 2), and a shell-gas p_{O_2} of 54.6 mmHg (Fig. 3). The same animal, with a blood volume of 90 ml (ref. 2) equally distributed between a 30% O_2 -saturated venous and 70% O_2 -saturated arterial compartment, and a maximal blood-oxygen-carrying capacity of 2.3 ml O_2 per 100 ml (ref. 8), would have 1.0 ml O_2 available in the blood. With this available blood-oxygen store, a 450-g *Nautilus*, with a resting $\dot{V}O_2$ (oxygen consumption) of 0.28 ml O_2 per min (Fig. 1), could either descend into an anoxic zone or retreat into its shell, and have enough oxygen to sustain aerobic metabolism for ~3.5 min. The impact of lowering its metabolic rate to the levels seen during severe hypoxia (Fig. 1) would be to extend this time to ~50 min. However, if the rates of O_2 transfer between shell gas and blood were high enough to support the metabolic rate, this could extend the time by up to 6 h. An upper estimate of the rate that oxygen might feasibly be metered from the shell can be made by assuming that O_2 would diffuse through the wall of the siphuncle as rapidly as through water. The volume of oxygen that could so diffuse can be calculated by the equation, $\Delta V O_2 = -DA(\Delta x/\Delta y)\Delta \text{time}$, where D is the diffusion coefficient for O_2 in water ($25 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), A is the total siphuncular surface area (13 cm^2 ; ref. 5) and $\Delta x/\Delta y$ is the concentration gradient between shell gas and blood, Δy being the diffusion barrier thickness (0.027 cm; ref. 5). At the 31‰ salt concentration of cameral

FIG. 2 a, Ventilation rate and cardiac output measured at the time of blood sample withdrawal. b, Arterial and venous p_{O_2} measurements. Data are from an individual animal undergoing progressive hypoxia in a closed respirometer, as in Fig. 1. Chronic indwelling catheters were implanted 24 h before the experiments to enable repetitive sampling of arterial and mixed venous blood from undisturbed animals as before². c, Simultaneously recorded arterial and venous p_{O_2} levels for 6 animals undergoing progressive hypoxia as in Fig. 1. In the latter stages of the experiment, when hypoxia was most severe, ventilation rate was highly intermittent in all animals. In four of the six animals studied, repetitive blood samples taken to coincide with the periods before and after the most prolonged of these respiratory pauses, revealed that the venous p_{O_2} occasionally exceeded that of simultaneously drawn samples of arterial blood (note the reversed arterial–venous p_{O_2} gradients). p_{O_2} measurements were made on blood samples drawn in Hamilton gas-tight syringes using Radiometer E-5046 electrodes and D616 cuvettes thermostatted to 18 °C. Electrodes were preconditioned before every measurement to the approximate blood gas levels as detailed earlier¹⁹, and calibrated with precision gas mixtures before and after each measurement, with linear interpolation being used to account for any small drift.



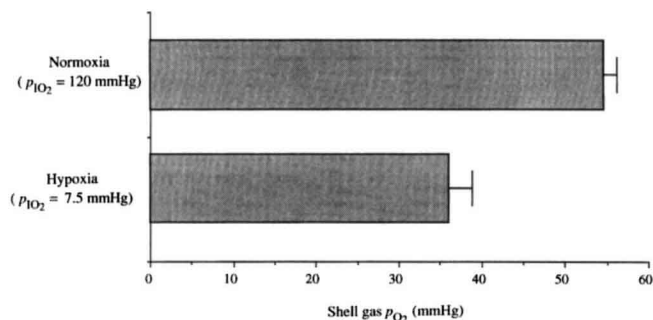


FIG. 3 Partial pressures of oxygen P_{O_2} in cameral gas samples taken from 5 normoxic animals and in the same animals after they had been subjected to 8 h of hypoxia, as in Fig. 1. The day before an experiment, gas sampling ports were drilled into two of the most recently formed shell chambers. Gas-tight chromatography septa were then immediately sealed in place over each hole using cyanoacrylate glue. Gas samples of 0.5 ml were taken with Hamilton gas-tight syringes and measured with Radiometer electrodes and cuvettes as in Fig. 2. Measurements of cameral gas p_{O_2} in normoxic animals made 12–16 h before the experiments began were not significantly different (Student's paired t-test. $P < 0.05$) from the samples taken immediately before the exposure of animals to hypoxia, so we can be reasonably certain that the decline in p_{O_2} during 8-h hypoxia was due directly to the environmental O_2 lack, and not to a time-dependent fall in p_{O_2} in normoxia. p_{O_2} , inspired P_{O_2} .

fluid⁵ and a shell p_{O_2} of 54.6 mmHg (Fig. 3), the cameral fluid would contain $(54.6/155) \times 5.98 \times 10^{-3} = 2.107 \times 10^{-3}$ ml O_2 per ml fluid. Therefore, the ΔV_{O_2} (ml) = $25 \times 10^{-6} \times 13 \times (2.107 \times 10^{-3}/0.027) \times 3600 = 0.09$ ml O_2 h⁻¹ for a 450-g *Nautilus*. At the measured hypoxic metabolic rate of 1.19 ml O_2 h⁻¹, the fraction attributed to the shell O_2 would be ~8%. On the other hand, the data in Fig. 3 reveal a mean p_{O_2} decline in the shell gas of 18.5 mmHg (2.4 ml O_2) over 8 h, which is equivalent to a transfer rate of 0.3 ml O_2 per h, and to a fractional contribution (0.3/1.19) of 25%. Even greater contributions may be possible at *Nautilus* deep-water habitat temperatures of 8–9°C (ref. 7) where, with a Q_{10} approximating 3–4 (refs 9,10), the shell O_2 store could sustain a large proportion of the animal's hypoxic metabolic rate.

Nautilus is unique amongst the cephalopod molluscs in having the capacity to survive prolonged periods of low O_2 levels. It

accomplishes this by sharing the mechanisms used by other 'good' facultative animal anaerobes^{3,4}, namely a marked aerobic metabolic rate suppression and a modest reliance on anaerobic energy production. However, the hypometabolic, hypoxic *Nautilus* may well live up to its reputation as a zoological oddity, as much in function as in form, if we are correct in suggesting the ability to use a superficial circulation to load its blood with O_2 at the venous side and to utilize, albeit slowly, the oxygen contained in its buoyancy chambers to help sustain aerobic metabolism. To speculate whether a similar suite of hypoxic defence mechanisms contributed to the success of ectocochleate forms, by enabling them to exploit the O_2 -deficient waters of the Palaeozoic for refuge or feeding^{2,11,12,13}, adds to the debate over which features were to remain shared after the early divergence of the nautiloid and ammonoid lineages¹⁴. □

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A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*

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A PHEROMONE-INDUCED neurosecretory pathway in *Caenorhabditis elegans* triggers developmental arrest and an increase in longevity at the dauer diapause stage. The gene *age-1* is required for non-dauer development and normal senescence. *age-1* encodes a homologue of mammalian phosphatidylinositol-3-OH kinase (PI(3)K) catalytic subunits. Lack of both maternal and zygotic *age-1* activity causes dauer formation, whereas animals with maternal but not zygotic *age-1* activity develop as non-dauers that live more than twice as long as normal. These data suggest that phosphatidylinositol signalling mediated by AGE-1 protein controls lifespan and the dauer diapause decision.

TABLE 1 Concentrations of anaerobic metabolic end-products in normoxic and hypoxic *Nautilus*

	Adductor muscle	
	Octopine	Succinate
Normoxia	0.6 ± 0.1	1.2 ± 0.2
Hypoxia	7.2 ± 1.3	1.8 ± 0.4

Adductor muscle was taken from freshly killed animals and rapidly frozen in liquid nitrogen for subsequent analysis of anaerobic end-products as before¹⁵. Values are means ± s.e.m. ($n = 6$) for both normoxic animals at rest and for those sampled after 500 min progressive hypoxia. Octopine and succinate concentrations are given in μ mol per g wet weight. In molluscs, the terminal dehydrogenase of anaerobic glycolysis is that of octopine¹⁶ which is formally analogous to lactate dehydrogenase in vertebrates¹⁷. Succinate represents another alternative anaerobic fermentation pathway in many molluscs^{17,18}. Estimates of ATP turnover, calculated from normoxic and hypoxic aerobic metabolic rates (168 and 11.9 μ mol per kg ATP per min, respectively) and from the accumulation of octopine and succinate^{17,18} in hypoxic animals (7.1 μ mol per kg ATP per min) indicate that accelerated glycolytic flux makes up only 4.5% of the O_2 deficit. Anaerobic ATP turnover was calculated assuming that the metabolite accumulation in adductor muscle reflected that of the total muscle mass of a 1-kg animal, having a shell weight of 333 g and a muscle mass of 230 g (40% of flesh weight).