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REVIEW

Structure, function and evolution of the gas exchangers: comparative perspectives

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Abstract

Over the evolutionary continuum, animals have faced similar fundamental challenges of acquiring molecular oxygen for aerobic metabolism. Under limitations and constraints imposed by factors such as phylogeny, behaviour, body size and environment, they have responded differently in founding optimal respiratory structures. A quintessence of the aphorism that 'necessity is the mother of invention', gas exchangers have been inaugurated through stiff cost–benefit analyses that have evoked transaction of trade-offs and compromises. Cogent structural–functional correlations occur in constructions of gas exchangers: within and between taxa, morphological complexity and respiratory efficiency increase with metabolic capacities and oxygen needs. Highly active, small endotherms have relatively better-refined gas exchangers compared with large, inactive ectotherms. Respiratory structures have developed from the plain cell membrane of the primeval prokaryotic unicells to complex multi-functional ones of the modern Metazoa. Regarding the respiratory medium used to extract oxygen from, animal life has had only two choices – water or air – within the biological range of temperature and pressure the only naturally occurring respirable fluids. In rarer cases, certain animals have adapted to using both media. Gills (evaginated gas exchangers) are the primordial respiratory organs: they are the archetypal water breathing organs. Lungs (invaginated gas exchangers) are the model air breathing organs. Bimodal (transitional) breathers occupy the water–air interface. Presentation and exposure of external (water/air) and internal (haemolymph/blood) respiratory media, features determined by geometric arrangement of the conduits, are important features for gas exchange efficiency: counter-current, cross-current, uniform pool and infinite pool designs have variably developed.

Key words adaptation; air; evolution; gills; lungs; oxygen; respiration; water.

Introduction

> 'When something is designed, decisions are necessarily made. Deliberate progress cannot proceed without choices – as whether a part goes to the right or left of another part, whether a component is larger or smaller. In some cases, a clear historical record documents for us the evolutionary design process that brought us the thing that we contemplate. Looking into that record in detail can help us understand why certain things are the way they are and, perhaps more importantly, help us understand how things in general come to take the forms that they do.' Petroski (2000)

The field of comparative respiratory biology is progressing rapidly. Superior analytical methods and more novel approaches are moving the scope to fascinating horizons. The inventiveness continues to confound students as well as specialists in the area. The expansion of the information base is empowering investigators to better synthesize, rationalize, reconcile, redefine, consolidate and verify some of the earlier anecdotal views and inferences. Occasional reviews of the advances

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†This paper is dedicated to (Emeritus) A. S. King (University of Liverpool) for his able guidance during my faltering years and his continued friendship.

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help outline new grounds, formulate new concepts and evoke further inquiry. Moreover, such surveys of the literature should promote communication among investigators, preventing costly duplication of effort.

In biology, few processes are as important for life as respiration. August Krogh (1941) termed the critical need for molecular oxygen simply 'the call for oxygen' while Laitman et al. (1996) asserted that 'the acquisition and processing of oxygen and its byproducts is the primary mission of any air-breathing vertebrate'. Respiration, the process by which oxygen is contracted from an external environment and delivered to tissue cells for energy production, entails a complex arsenal of structural components and co-ordinated physiological, biochemical and behavioural processes. While animals will exist for weeks lacking food and days without water, oxygen is needed continuously.

Max Kleiber (1961) termed oxygen 'the fire of life'. Unlike metabolic substrates like carbohydrates and fats that can strategically be stored in large quantities in the body and used as need arises, oxygen has to be continually contracted from outside. The efficiency of obtaining and utilizing oxygen for metabolic purposes attests to the demands placed upon an animal by the environment it lives in and the activities it performs. Respiratory efficiency bespeaks of the speed at which an animal can mobilize its resources to meet the selective pressures experienced. The correlation between an animal's environment and its respiratory needs is a very intimate one.

Gas exchangers have developed tractably to meet specific metabolic requirements of whole organisms in different physiological states and ecological settings. The essence of the morphology of a gas exchanger can best be understood by discerning the intercourse between the habitat and the organism itself. It is axiomatic that the evolution of complex, energetic animal life should have paralleled development of efficient respiratory organs.

The designs of the gas exchangers display structural properties that natural selection has rigorously tested, refined and genomically conserved. Protracted cost–benefit analyses have elicited transactions that have entailed certain trade-offs and compromises. From the tenets of optimal design and symmorphosis in biology (e.g. Weibel et al. 1998), the various constructions of the gas exchangers must impart certain functional advantages and should incur least cost to develop, operate and maintain.

Holistically, structure encompasses the qualitative and quantitative attributes of a biological entity. Altering the proportions and positions of the constitutive structural components generates new polarities, conferring different functional capacities. Through apt sizing and arrangement of the structural elements, the designs of gas exchangers have adaptively been continually fashioned for optimal acquisition of oxygen. At certain junctures in the evolutionary continuum, e.g. when the metabolic needs surpassed those that could be adequately sustained by the default gas exchanger or the atmosphere changed drastically, especially regarding levels and availability of oxygen, adaptive structural changes occurred to enhance respiratory efficiency. With regard to the evolution of terrestrial vertebrates, fairly chronologically, quantum leaps in morphological and physiological transformations of the gas exchangers and the respiratory processes happened at:

1 change of anaerobiotic to aerobiotic life, i.e. the evolution of aerobic biochemistry;

2 accretion of diffusion-dependent unicells into multicellular organisms, complex states that obliged elaboration of convective and perfusive systems for effective delivery of oxygen to far removed tissue cells;

3 formation of a closed circulatory system from an open one, a design that enhanced return of blood to the heart and delivery of oxygen all over the body;

4 evolution of metal-based carrier pigments that improved oxygen uptake and transfer by blood/haemolymph;

5 formation of invaginated respiratory organs ('lungs'), a transition that was requisite for water conservation on the desiccating terra firma;

6 physical translocation from water to land, a change that accentuated utilization of lungs as respiratory organs while de-emphasizing that of the gills;

7 development of double circulation from a single one, a transformation that granted efficient delivery of oxygen to the tissues;

8 shift from buccal-force-pumping to suctional breathing, a more economical and efficient means of transport of air to the lung;

9 progression from ectothermic-heterothermy to endothermic-homeothermy, a high-level metabolic state that required evolution of efficient respiratory organs;

10 capacity for highly energetic lifestyles (e.g. flight), performances that exacted singularly efficient respiratory organs.

Formulating the transformations that have occurred and trajectories followed in the evolution of gas exchangers, soft tissues that are rarely fossilized, is a rather uncertain enterprise. The only vertebrate lungs that have ever been fossilized are those of the Devonian placoderm, *Bothriolepis* (Denison, 1941), particularly poor representatives of the vertebrate lungs. With due precautions, however, the progress can be reasonably pieced together by studying animals that:

1 subsist in unique habitats, especially regarding availability or lack of oxygen;

2 display unique behavioural activities and metabolic capacities;

3 have adopted transitional respiration, i.e. use various gas exchangers to procure oxygen from air and water;

4 are at different phylogenetic levels of development and have thus committed different infrastructural resources towards construction of gas exchangers;

5 show unique developmental transformations during their life histories, with shifts in respiratory pathways, strategies and mechanisms.

While highlighting the factors that have prescribed the various designs of the gas exchangers, this brief survey of a very broad subject outlines the diversity of architecture of the evolved respiratory organs in different animal taxa.

Oxygen: a paradoxical molecule

The morphologies of gas exchangers have been profoundly influenced by the presence in the biosphere (in substantial quantities) of the highly reactive oxygen molecule. An 'excretory' product of photosynthesis by plants and cyanobacteria, oxygen first appeared in significant quantity some 2 billion years ago (e.g. Owens et al. 1979). Its paucity in most of the Precambrian is conceived to have consigned life to simple, anaerobic prokaryotic biota (e.g. Knoll, 1996). The so-called 'Cambrian Explosion', an event marked by unprecedented adaptive radiation and morphological diversification of animal life (e.g. Wray et al. 1996), is associated with upsurge of oxygen at the Precambrian–Cambrian boundary (e.g. Chapman & Schopf, 1983).

The intensifying level of molecular oxygen in the Cambrian may help explain the short period (in evolutionary terms) of 700 million years that it took for the change of eukaryotic unicells to multicellular organisms (Metazoa) compared with the over 2 billion years that passed for the aerobic eukaryotes to develop from anaerobic prokaryotes (e.g. Gould, 1994). Aerobic metabolism granted an efficient process of energy production that was vital for building, servicing and maintaining infrastructural integrity of complex cells and tissues. The very high level of oxygen from the Devonian to the Permian, a hyperoxic episode during which the level of atmospheric oxygen rose to a high of 35% (compared to the present one of 21%) (e.g. Graham et al. 1995), allowed development of exceptionally large animals such as the giant dragonfly like *Meganeura* that reached a body length of 60 cm (e.g. Krogh, 1941).

Despite its now quintessential role in the survival of complex animal life, an anoxic (reducing) environment was obligatory for chemical formation of incipient life (e.g. Chang et al. 1983). It is therefore anomalous that subsequently life became intricately dependent on it. Three factors may help explain this paradox:

1 the mounting level of oxygen in the biosphere left the emerging animal life with no alternative but to protect itself from molecular oxygen or face inevitable annihilation from its oxidative reactions;

2 the 'small' size of the molecule and hence the high intracellular diffusivity and correct redox potential of oxygen strongly inclined it for utilization as an electron acceptor in the energy production pathways of the tricarboxylic cycle where it mops up protons (H^+) to form water, an innocuous and important chemical factor for life;

3 aerobic metabolism conferred great advantages since it yielded 20 times more free metabolic energy than was achievable through anaerobic pathways.

Utilization of oxygen is accompanied by great danger. This ensues from reactive oxygen species (RORs) [e.g. the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$)], biochemically deleterious molecules with an unpaired electron oxygen, factors highly toxic to carbon-based life (e.g. Filho et al. 2000; Hermes-Lima et al. 2001). The assault by the RORs on the DNA, proteins and other macromolecules is profound. It is estimated, for example, that about 2–3% of oxygen taken up by aerobic cells results in production of O_2^- radical and H_2O_2 (e.g. Chance et al. 1979); about 10^{12} oxygen molecules are handled by a rat cell daily, generating about 2×10^{10} (i.e. 2%) O_2^- and H_2O_2; about 9×10^4 attacks on the DNA per day per cell occur in a rat (Fraga et al. 1990); and RORs are responsible for 10 000 or so DNA base modifications per cell per day (Ames et al. 1993).

The formation of RORs inside cells constitutes a serious threat, particularly to the functional and structural integrity of the genome (e.g. Epe, 1995). It is envisaged that control of oxygen toxicity could have necessitated the evolution of the nucleus and the nuclear membrane in the eukaryotic cells to minimize assaults by RORs (e.g. Margulis, 1981): the nucleus constitutes an anoxic and fairly safe location for the DNA. The mitochondrial DNA that is relatively more exposed to oxygen toxicity has more than 10 times the level of oxidative DNA damage than does the nuclear one (e.g. Dyer & Ober, 1994).

Water and air as respiratory media: consequences on the design of gas exchangers

In the biosphere, over the biological range of temperature and pressure, water (a liquid) and air (a gas) have been the only two naturally occurring respirable fluids. Regarding acquisition of oxygen, animals have had very few choices except to adapt to utilizing one or the other and in rare cases both. Prescribed by the different physicochemical properties of water and air, gills and lungs have evolved for respiration in the respective media. The structure of the two types of gas exchangers differ so much that gills cannot function efficiently in air and neither can the lungs in water.

The common expression 'like a fish out of water' bespeaks of the well-known inability of most fish and other aquatic life-forms to survive and acquire adequate amount of oxygen from air through gills. In teleosts, for example, secondary lamellae (Fig. 1A) and gill filaments in the crustacean gills (Fig. 2B), closely packed, delicate, leaf-like respiratory units, dry out and become impermeable to oxygen. Furthermore, they cohere due to surface tension and collapse under their own weight. This reduces the respiratory surface area, creates large diffusion distances in the lamellae and increases branchial vascular resistance. The animal becomes anoxic and hypercapnic. It eventually succumbs to asphyxia, inspite of being exposed to a respiratory medium (air) richer with oxygen.

Regarding the process of breathing water using lungs, owing to the high viscosity of water, the ventilatory rate is much slower (e.g. Leith & Mead, 1966). Moreover, inhaled into a lung, liquids physically destroy alveoli (e.g. Curtis et al. 1993), dissolve and mechanically displace the surfactant (e.g. Lewis et al. 1993), osmotically interfere with the composition of the body fluids, cause pathological changes such as interstitial oedema and produce intrapulmonary froth and atelectasis upon re-exposure to air (Blenkarn & Hayes, 1970). Furthermore, macrophages are lost (e.g. Huber & Finley, 1965) and airway constriction increases (e.g. Yager et al. 1989).

Compared with air breathing, water breathing is the more ancient mode of respiration. As a respiratory medium, air is a more cost-effective respiratory fluid: water is 50 times more viscous than air; the concentration of dissolved oxygen in water is about one-thirtieth that in air; the rate of diffusion of oxygen in water is lower by a factor of 8×10^3 compared with that in air; and the capacitance coefficient, i.e. increment of concentration per increment in partial pressure of oxygen, in water is 30 times lower in air. In saturated water, at 20 °C, 1 mL of oxygen is contained in 200 g of water while 1 mL of oxygen is present in 5 mL of air (mass, 7 g). All other conditions being equal, owing to greater viscosity of water, compared with air breathing, water breathing requires more energy to procure an equivalent amount of oxygen. An octopus, *Octopus vulgaris*, for example, ventilates 17 L of water for each mmol of oxygen consumed (Dejours et al. 1970).

Fundamental principles in the design of gas exchangers

The design and respiratory efficiency of gas exchangers is closely matched to oxygen needs of an animal. Volant animals, e.g. birds, insects and bats, animals that have high metabolic capacities, have the most efficient respiratory organs (e.g. Weis-Fogh, 1967; Maina & King, 1984; Maina et al. 1989a, 1991; Maina, 2000a). Including gas exchangers, biological structures are dynamic, composite, multifunctional entities. In such cases, founding optimal morphological states and physiological capacities invokes sound analysis of alternative strategies, cost and benefits. Transaction of compromises and trade-offs is invoked. Modern gas exchangers are products of a protracted evolutionary development: they give us an opening, though nebulous, of conceiving what may have evolved under different circumstances in the past.

The basic similarities in the designs of vertebrate gas exchangers (e.g. Maina, 2000b) can be attributed: (1) to the fact that they arose from a common primordial structure of which the primary design has been conserved; and/or (2) that gas exchange is such an important

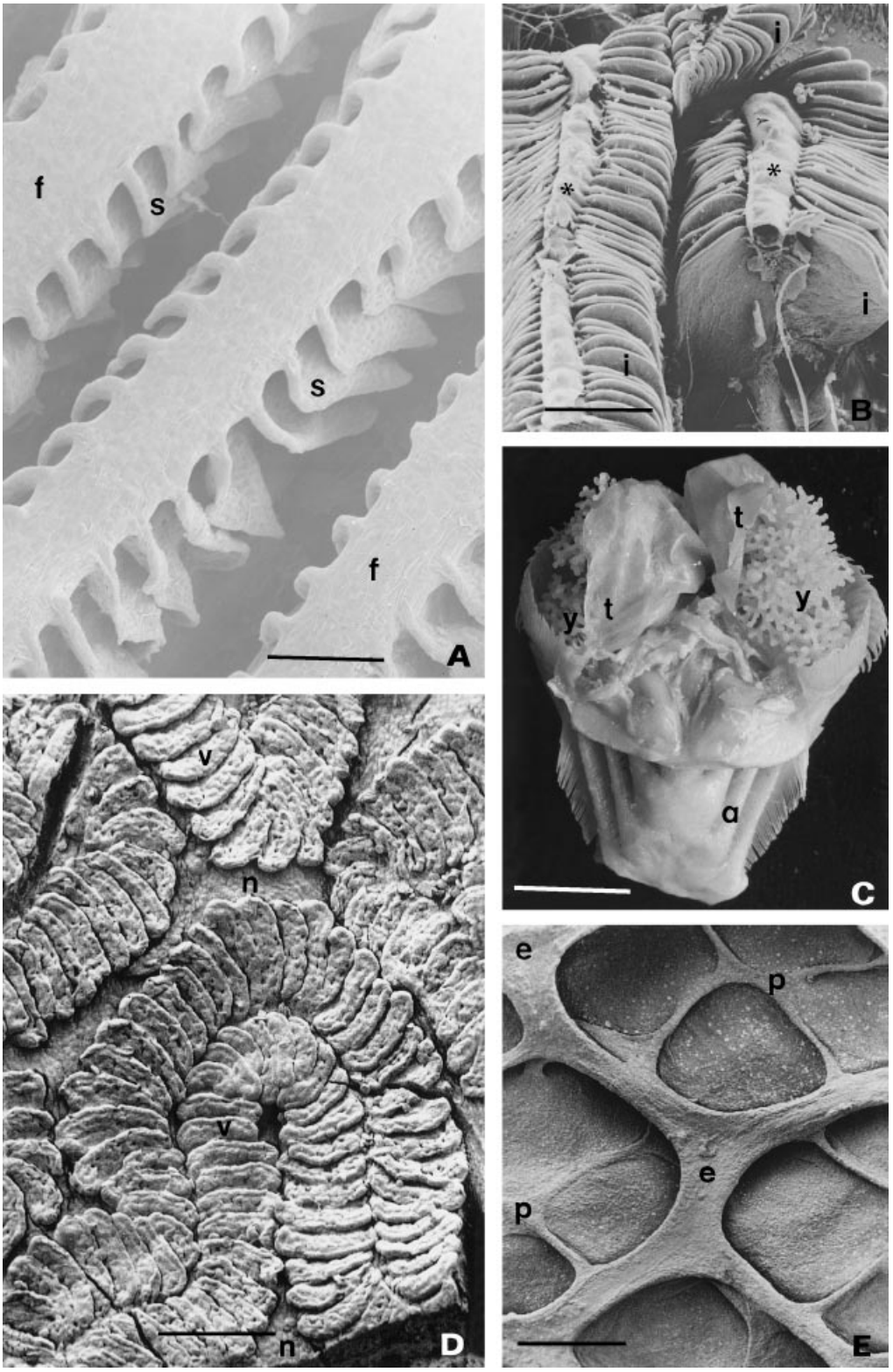

Fig. 1 (A) Gill filaments (f) of *Alcolapia grahami* showing secondary lamellae (s) emanating from both sides of gill filaments. Scale bar, 0.4 mm. (B) Gills arches (*) and gill filaments (i) of a crab, *Potamon niloticus*. Scale bar, 0.3 mm. (C) Respiratory organs of the catfish, *Clarias mossambicus*, showing gill arches (a), labyrinthine organs (y) and suprabranchial chamber membranes (t). Scale bar, 10 mm. (D) Transverse capillaries (v) lining the suprabranchial chamber membranes of *Clarias mossambicus* and tracts occupied by mucous secretory cells (n). Scale bar, 10 μm. (E) Internal subdivision of the lung of the savanna monitor lizard, *Varanus exanthematicus*, showing primary septa (e) that line the central air space and peripheral septa (p). Scale bar, 0.1 mm.

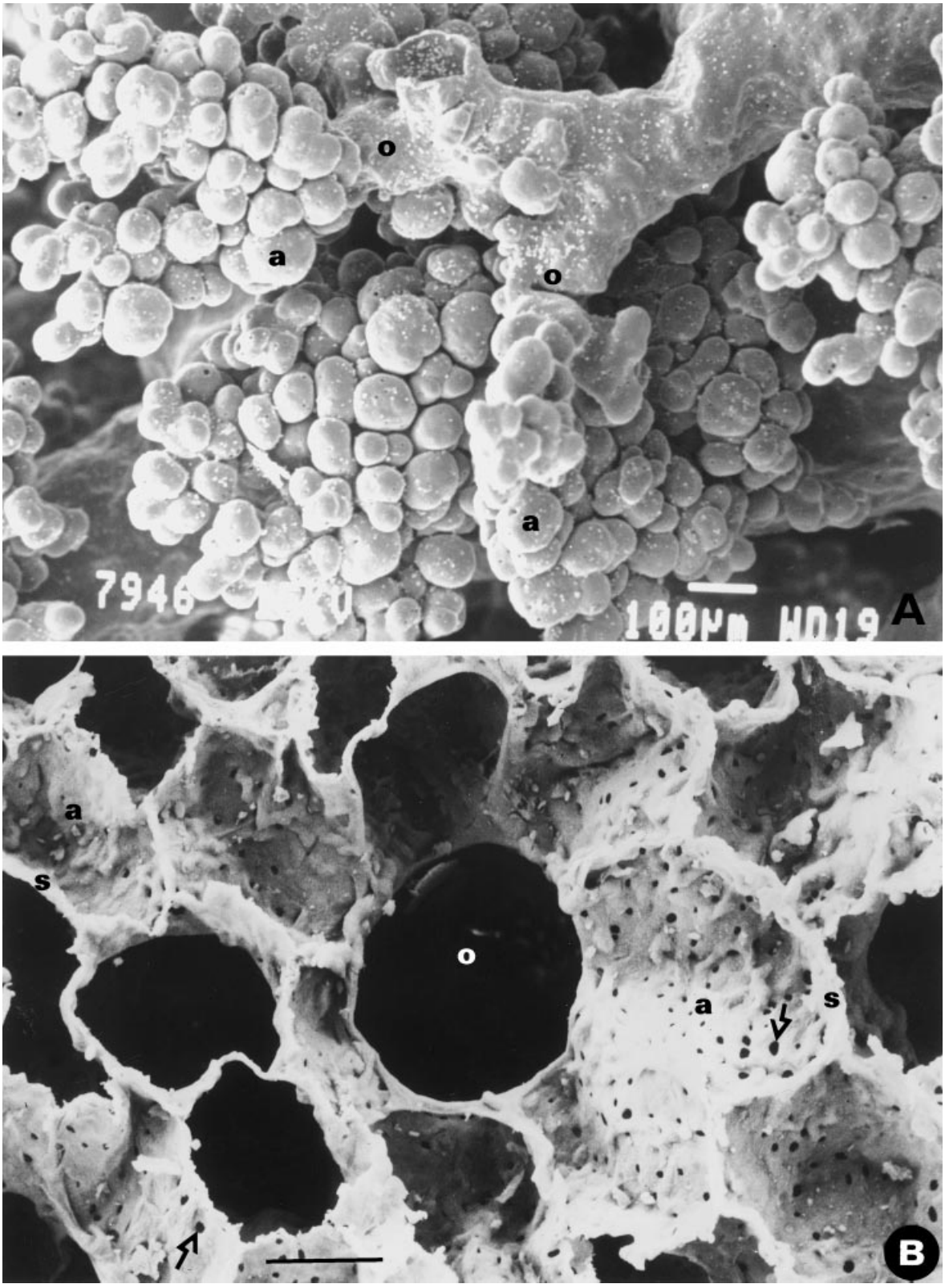

Fig. 2 (A) Grapefruit-like formation of alveoli (a) on the terminal parts of respiratory bronchioles (o) of the lung of a pig, *Sus scrofa*. Scale bar, 100 μm. (B) Lung of the lesser bushbaby, *Galago senegalensis*, showing respiratory bronchioles (o) giving rise to alveoli (a) that are very well vascularized. s, interalveolar septa; arrows, interalveolar pores (the pores of Kohn). Scale bar, 40 μm.

process for life that it has prescribed an essential biological construction of the structures engaged in acquisition of molecular oxygen. For example, contrary to the belief at the turn of this century (e.g. Haldane, 1922) that the transfer of oxygen across the vertebrate lung occurred by active flux, it is now clear that after about 2 billion years of evolution of aerobic metabolism, the transfer of molecular oxygen in all the evolved gas exchangers still occurs by passive diffusion: only ways have changed – the means have endured. Evolutionary change rarely occurs through radical deconstruction but rather by parsimonious 'improvement' upon a default structure.

Lungs are thought to have evolved as an adaptation to hypoxic or anoxic conditions in the early hydrosphere. However, air breathing did not develop *ipso facto* for terrestrial habitation but was unquestionably an important and necessary adaptation for successful survival in the water. Shift from breathing water to air called for profound changes in the structure and function of gas exchangers. The extant bimodal breathers, animals that commute between water and air, are analogues (frozen in time) of the transitory stage.

Air breathing has evolved in many animal taxa (e.g. Randall et al. 1981; Maina, 1998). To a remarkable extent, numerous fish display evolution of air breathing (e.g. Singh, 1976). As many as 370 species are known to breath air (e.g. Graham, 1994). Air breathing has become so perfected in some fish that they succumb on forced submersion. Using accessory respiratory organs, e.g. suprabranchial chamber membrane and labyrinthine organ for respiration (Fig. 1C,D), the Indian catfish *Clarias batrachus*, which subsists in shallow derelict waters, 'comes out at night to feed on earthworms' (Dehadrai & Tripathi, 1976). Other Indian species such as the climbing perch, *Anabas testudineus*, and the Cuchia eel, *Amphipnous cuchia*, are highly terrestrial, spending much of their time out of water (e.g. Munshi & Hughes, 1992). *Anabas* acquires about 54% of its oxygen needs from air (Hughes & Singh, 1970). In most air-breathing fish, wide gill filament and secondary lamellar spacing prevents coherence and collapse (e.g. Graham, 1973). In *Clarias mossambicus*, the extremely thin blood–gas barriers of the accessory respiratory organs provide a high diffusing capacity for oxygen compared with the gills (e.g. Maina & Maloiy, 1986).

Nature has been particularly inventive in the evolution of gas exchangers. The foremost factors that have jointly prescribed the design of the gas exchangers include:

1 respiratory medium utilized;
2 habitat occupied;
3 phylogenetic level of development achieved;
4 body size;
5 metabolic capacity and lifestyle pursued.

The permutative nature of the process is illustrated in the fact that respiratory efficiency cannot be reliably predicted from any one single factor but rather from consideration of the spectrum of the roles performed. For example, while humans may stand at the pinnacle of evolutionary development as regards the advancement of the brain, the human lung does not present the pre-eminent design in animals.

In its most elementary form, a gas exchanger is a tissue barrier across which flux of oxygen and carbon dioxide occurs under partial pressure gradients. Such a design is presented in its entirety in the extant unicells, where intracellular and extracellular compartments are separated by only a cell membrane. While the transfer of oxygen in simple life occurs entirely by diffusion across the surface, in the more complex animals, it is confined to specialized sites where an assemblage of tissue barriers and compartments from the respiratory medium-haemolymph/blood interface to the mitochondria are crossed. Conspicuous gas exchangers cannot be delineated below the level of molluscs and arthropods. Above the basic design, gas exchangers display certain or all of the following basic morphological features:

1 evagination or invagination from the body surface;
2 stratification or compartmentalization, means by which an extensive surface area is generated in a limited space;
3 thin partitioning between internal and external compartments, a property that promotes flux of respiratory gases;
4 vascularization, an attribute that increases the volume of blood exposed to external respiratory medium;
5 geometric organization of the structural components, characteristics that determine the interaction between the respiratory media.

Evagination and invagination of gas exchangers

Evolution of invaginated gas exchangers was requisite for successful terrestrial habitation: water loss across an extensive respiratory surface area was minimized. Gills

are evaginated gas exchangers while lungs are invaginated ones. In animals, generally, no other organ is in more contact with an external environment than the gas exchanger. If, for example, the mature human lungs, of which the alveolar surface is 143 m^2 (Gehr et al. 1978), were designed like gills (i.e. they were evaginated and exposed to air) even in a moderately desiccating environment, water loss would be about 500 L per day, a value about 1000 times more than normal loss (McCutcheon, 1964).

While evaginated gas exchangers can be ventilated continuously and unidirectionally through buccopharngeal pump or ram ventilation, by the very nature of the lungs developing as invaginated organs, thus having a narrow entry/exit point to the ambient milieu, they can only be ventilated tidally, i.e. bidirectionally (= in-and-out). Inescapably, dead space is created in the major air-conducting passages, greatly affecting respiratory efficiency. In a resting person where the dead space is about 140 cm^3, about 28% of the 500 cm^3 of the inhaled air (tidal volume) does not reach the respiratory region of the lung. This is one of the many examples of the trade-offs and compromises, mentioned above, that have occurred in the evolution of gas exchangers: in this particular case, water conservation was achieved at the expense of respiratory efficiency.

Stebbins (1984) observed that 'the only law that holds without exception in biology is that exceptions exist for every law'; in birds, the gas exchange tissue of an invaginated respiratory organ, the lung – air sac system, is ventilated continuously and unidirectionally by synchronized action of air sacs (e.g. Scheid, 1979; Fedde, 1980), more like how evaginated fish gills are efficiently ventilated by the buccal–opercular pump. Similarly, in the insectan tracheal system, a gas exchanger formed through ectodermal invagination (e.g. Wigglesworth, 1972), air sacs and synchronized opening and closing of spiracles allows continuous unidirectional air flow (e.g. Weis-Fogh, 1967).

Stratification and compartmentalization of gas exchangers

An effective gas exchanging organ must have external (air/water) and internal (blood/haemolymph) gas exchange media exposed to each other over an extensive area. In gills, large surface area is achieved by hierarchical structural design. In teleosts, four pairs of gill arches give rise to hundreds of gill filaments that in turn together generate thousands of secondary lamellae (Fig. 1A). In certain fish, e.g. *Trachurus mediterraneus*, the secondary lamellae themselves are further subdivided (Hughes & Mondolfino, 1983).

In lungs, a large surface area is produced by internal subdivision of the parenchyma, giving rise to narrow terminal gas exchange components, faveoli in the reptilian lung (Fig. 1E) and alveoli in the mammalian one (Fig. 2A,B). While, for example, a sphere with a volume of 1 cm^3 has a surface area of 4.8 cm^2, 1 cm^3 of the lung of the shrew, *Sorex minutus*, has an alveolar surface area of 2100 cm^2 (Gehr et al. 1980).

In the human lung, there are about 300 million alveoli of an average diameter of 250 μm (Weibel, 1963), giving an overall alveolar surface area of 143 m^2 (Gehr et al. 1978). The compact, inexpansile state of the avian lung (e.g. Jones et al. 1985) (Fig. 3A) has allowed more intense subdivision of the gas exchange tissue: the air capillaries (Fig. 3B,C) range in diameter from 8 to 20 μm (e.g. Duncker, 1974; Maina, 1982) while the blood capillaries (Fig. 3D) are smaller. In the compliant mammalian lung, the narrowest alveoli (~50 μm) were reported in the shrew and an unnamed species of bat by Tenney & Remmers (1963). In air-breathing vertebrates, the degree of subdivision of the lung increases from amphibian, reptilian, mammalian and avian lungs (Maina, 1998). The highest mass-specific respiratory surface areas in birds (87 $cm^2\ g^{-1}$) has been reported in the violet-eared hummingbird, *Colibri coruscans* (Dubach, 1981), and the African rock martin, *Hirundo fuligula* (Maina, 1984). In mammals, the highest mass-specific respiratory surface (138 $cm^2\ g^{-1}$) was reported in the lung of the epauletted fruit bat, *Epomophorus wahlbergi*, by Maina et al. (1982).

Increasing the internal subdivision and hence the respiratory surface area of the lung occurs at a cost: in a compliant lung, narrow terminal gas exchange components demand more energy to dilate on ventilation and have a high propensity of collapsing. Surfactant, a complex material consisting primarily of phospholipid material (dipalmitoylphosphatidylcholine), reduces surface tension, preserving stability of the narrow terminal respiratory units (e.g. Cochrane & Revak, 1991). A balance between maximization of respiratory surface area, ventilatory capacity, size of the terminal gas exchange components and overall respiratory efficiency must be established in every gas exchanger. The presence of surfactant on the surface of the air capillaries of the rigid avian lung is rather enigmatic. It

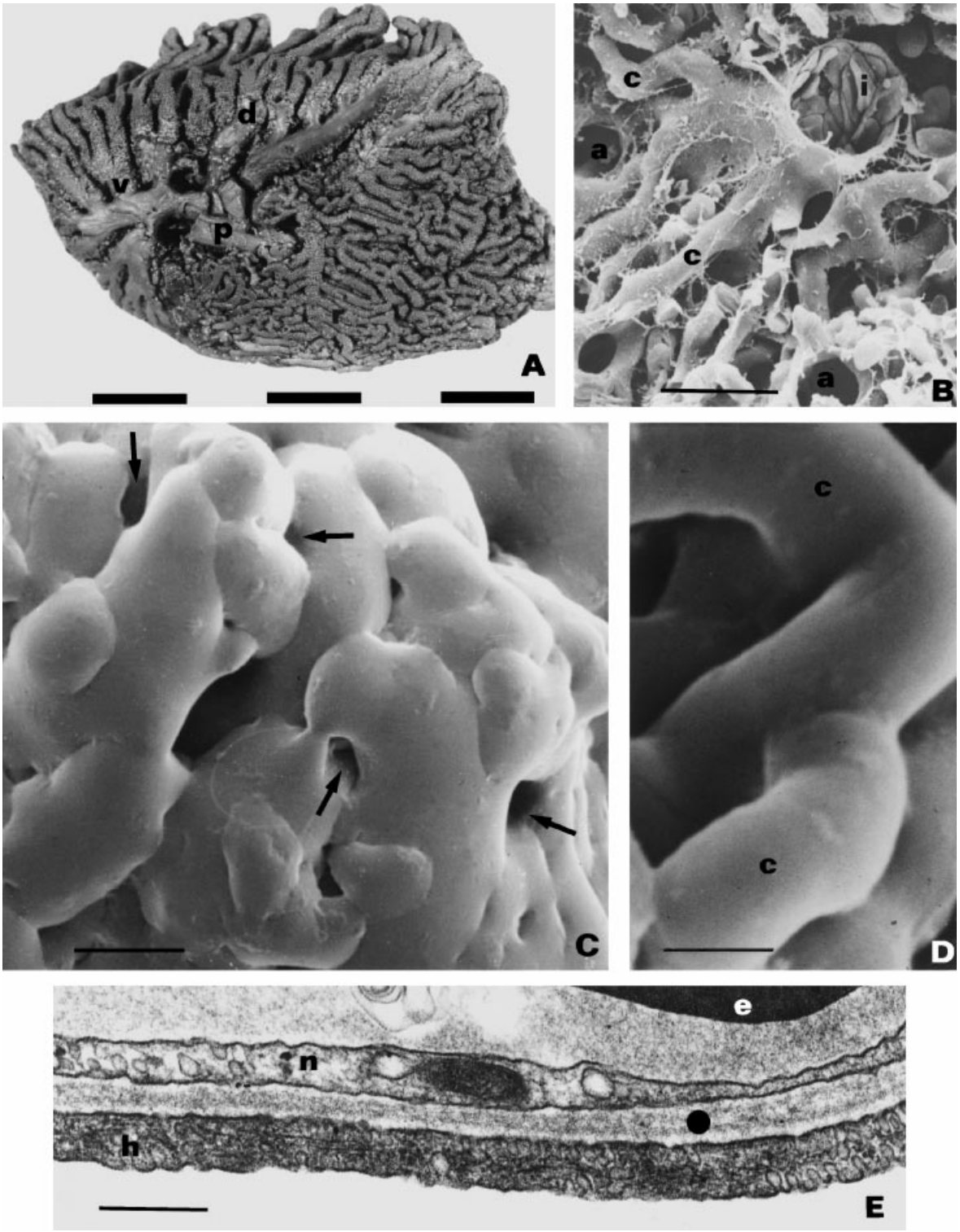

Fig. 3 (A) Medial view of the compact lung of the domestic fowl, *Gallus gallus* variant *domesticus*. p, primary bronchus; v, medioventral secondary bronchi; d, mediodorsal secondary bronchi; r, parabronchi. Scale bar, 1 cm. (B) An intraparabronchial artery (i) giving rise to blood capillaries (c) in the lung of the emu, *Dromiceus novaehollandiae*. a, air capillaries. Scale bar, 15 μm. (C) Air capillaries of the lung of the domestic fowl, *Gallus gallus* variant *domesticus*. They closely interdigitate with the blood capillaries (arrows). Scale bar, 10 μm. (D) Blood capillaries (c) of the lung of the domestic fowl, *Gallus gallus* variant *domesticus*, that closely interdigitate with the air capillaries (spaces). Scale bar, 12 μm. (E) Blood gas (tissue) barrier of the lung of the vervet monkey, *Cercopithecus aethiops*, showing a squamous epithelial cell (h) separated from an endothelial one (n) by a common basement membrane (●). e, erythrocyte. Scale bar, 0.3 μm.

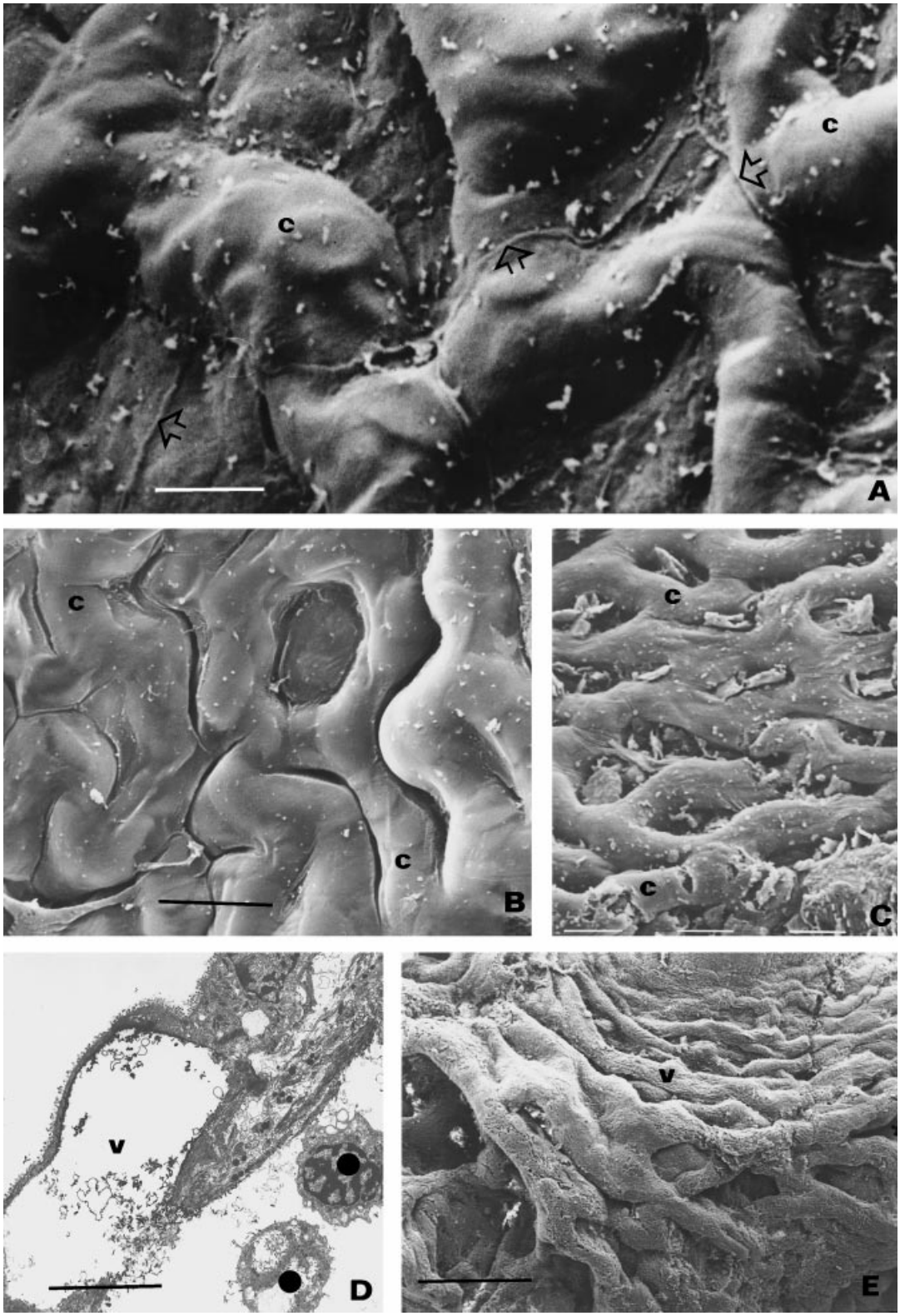

Fig. 4 (A) Vascularization and bulging of blood capillaries (c) on the alveolar surface of the lung of the lesser bushbaby, *Galago senegalensis*. Arrows, cell junctions of the squamous type I epithelial cells. Scale bar, 10 μm. (B) Vasularization of the faveolar walls of the lung of the snake, *Dendroapis polylepis*. c, blood capillaries. Scale bar, 8 μm. (C) Vascularization of skin of the tree frog, *Chiromantis petersi*. c, blood capillaries. Scale bar, 13 μm. (D,E) Blood vessels (v) on the surface of lung of the slug, *Trichotoxon copleyi*. ●, haemocytes. Scale bar, 8 μm (D); 20 μm (E).

may be involved in preventing transudation of blood plasma onto the respiratory surface.

Separation of internal and external respiratory media in gas exchangers

Respiratory media must be brought into very close proximity to each other to optimize gas exchange by passive diffusion. In vertebrates, the thickness of the blood–water/air (tissue) barrier increases from fish, amphibians, reptiles, mammals to birds (e.g. Hughes & Morgan, 1973; Goniakowska-Witalinska, 1978; Meban, 1980; Gehr et al. 1981; Perry, 1983; Maina et al. 1989a). In all taxa, the thickness of the barrier seems to have been optimized. While, for example, mammals span a remarkable range of body mass from 2.5 g (shrew) to the about 150 tonnes (whale), a factorial difference of 6×10^7, the thickness of the blood–gas (tissue) barrier of the lung of the shrew (Gehr et al. 1980) of 0.334 μm is comparable to that of 0.350 μm of the lung of the whale, *Balaena mysticetus* (Henk & Haldiman, 1990).

In birds, the violet-eared hummingbird, *Colibri coruscans* (7.3 g), has a blood–gas (tissue) barrier of 0.1 μm (Dubach, 1981) while that in a lung of a 40-kg ostrich, *Struthio camelus*, it is 0.56 μm (Maina & Nathaniel, 2001). In the avian lung, epithelial- and endothelial cells that constitute the blood–gas (tissue) barrier are separated only by a common basement membrane (e.g. Maina & King, 1982) (Fig. 3E). A common scheme that has occurred in the design of the blood–gas (tissue) is that of sporadic attenuation, particularly of the capillary endothelial cell (Maina & King, 1982) (Fig. 3E): in the lung of the graylag goose, *Anser anser*, for example, while the mean harmonic mean thickness of the barrier is 0.112 μm, certain parts of it are 0.050 μm. Intermittent reduction of the thickness of the blood–gas barrier maximizes the diffusing capacity of the lung without sacrificing its mechanical integrity.

Vascularization of the respiratory surface

The volume of blood in the pulmonary blood capillaries and its exposure to the external respiratory medium are fundamental to respiratory function. Increased vascularity and bulging of the blood vessels over the epithelial surface (Fig. 4A–E) both augment the capillary blood volume and the respiratory surface area. In the lungs of the lungfishes (Dipnoi), amphibians and many reptilian species (e.g. Hughes & Weibel, 1976; Perry, 1983; Maina & Maloiy, 1985, 1988; Maina, 1987a), blood capillaries are located on opposite sides of the intrapulmonary septa. The blood capillaries are exposed to air only on one side, forming a double capillary arrangement (Fig. 5A,B).

In mammalian lungs, blood capillaries are exposed to air on two sides (Fig. 5C), constituting a single capillary arrangement. The air and blood capillaries in the exchange tissue of the lungs of birds interdigitate profusely in three dimensions (Fig. 3B–D), generating a diffuse arrangement.

The capillary loading, the ratio between capillary blood volume and the respiratory surface area in a gas exchanger decreases from the double-, single- to diffuse capillary systems. In the lungfish, *Lepidosiren paradoxa*, the pulmonary capillary blood volume constitutes 3.5% of the total lung volume, in the rat lung 14%, and in birds 25%. In the human lung, forming an extremely thin sheet, 213 cm^3 of the pulmonary capillary blood is distributed over an area of 143 m^2 (Gehr et al. 1978).

Geometric arrangement of the structural components of gas exchangers

In addition to the structural refinements outlined above, the respiratory function of a gas exchanger is to a significant extent determined by the manner by which the respiratory media interact. Both the efficiency and the inadequacy intrinsic to the design of a gas exchanger is to a significant extent contributed by the geometric organization of the structural components (e.g. Maina, 1998). When the media run in opposite directions, the inducing design is termed 'counter-current'; when they meet perpendicularly, it is designated 'cross-current'; and when the external medium is held steady while fronting a gas exchanger or a gas exchanger is ventilated with a medium of which the partial pressure of oxygen (pO_2) is fairly uniform, the plans are termed 'infinite pool' and 'uniform pool', respectively (e.g. Piiper & Scheid, 1972). In the counter-current disposition of the fish gills, as venous blood is exposed to well-oxygenated (inspired) water, the pO_2 between the two media is very high. The remarkable efficiency of the counter-current system is essential for survival in a medium lacking oxygen and one more energetically expensive to respire.

An oxygen extraction ratio (EO_2), i.e. the ratio between the amount of oxygen taken up to that available in the

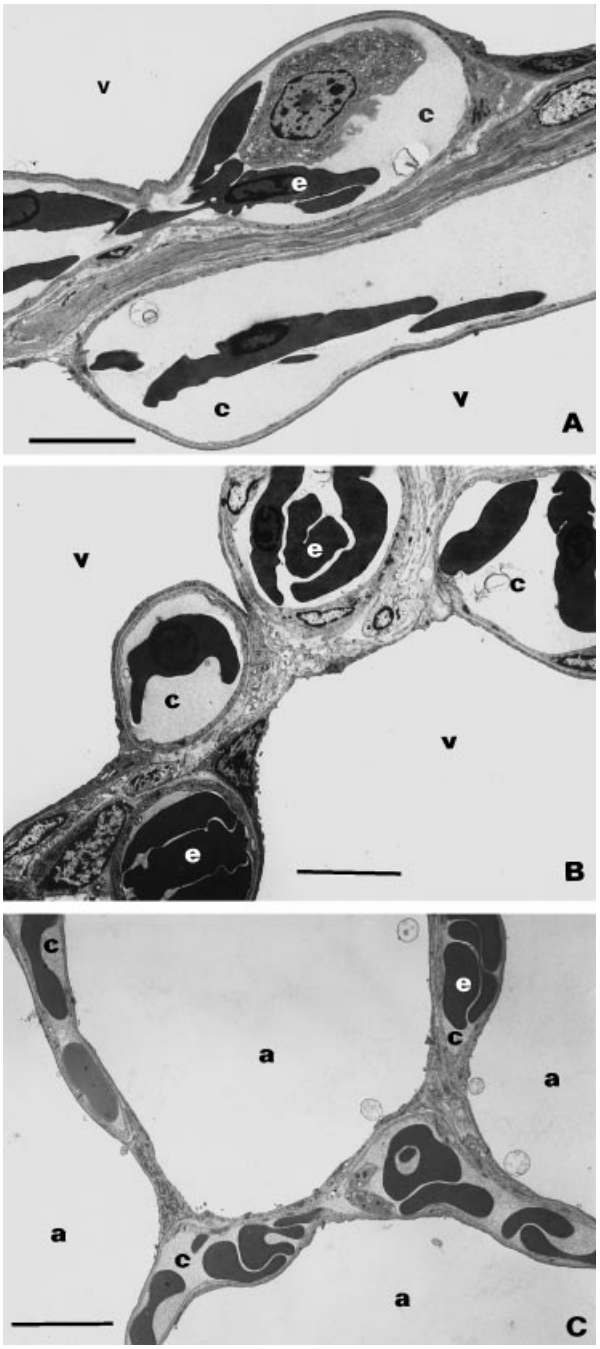

Fig. 5 (A,B) Double capillary arrangement on the interfaveolar wall of the lung of the black mamba, *Dendroapis polylepis*. c, blood capillaries; e, erythrocytes. v, faveoli. A: scale bar, 20 μm; B: scale bar, 13 μm. (C) Single capillary arrangement in the lung of the vervet monkey, *Cercopithecus aethiops*. c, blood capillaries; e, erythrocytes; a, alveoli. Scale bar, 10 μm.

inspired medium, as high as 92% has been estimated in the gills of the triggerfish, *Balistes capriscus*, by Hughes (1967): in the mammalian lung, EO_2 is *c*. 20% while values as high as 60–70% have been reported in some species of birds (e.g. Stahel & Nicol, 1988): values of EO_2 in air breathers are generally lower than those of fish and other water breathers (e.g. van Dam, 1954).

In the avian lung, the direction of the flow of the venous blood in the intraparabronchial arteries and finally in the blood capillaries is essentially perpendicular to that of the bulk air flow in the parabronchial lumen (Fig. 6A,B). This arrangement, termed 'cross-current', was experimentally confirmed by Scheid & Piiper (1972) and morphologically by, for example, Maina (1988).

Functionally, the arrangement of the blood capillary along the parabronchial lengths forms a serial, multi-capillary arterialization system (Fig. 6A,B). The cross-current system allows deoxygenated blood of uniform composition to be delivered to all parts of the parabronchi through many blood capillaries that arise from intraparabronchial arteries. Through protracted engagement between air and blood along unidirectionally and continuously ventilated parabronchi (mainly palaeopulmonic), the gas exchange efficiency of the avian lung is markedly enhanced. The total amount of oxygen in the arterialized blood returning to the heart from the lung ensues from a pooling effect of minute quantities of oxygen exchanged at infinitely many points (between blood capillaries and air capillaries) in the parabronchial gas exchange tissue. In certain conditions, e.g. under hypoxia or during exercise, the partial pressure of carbon dioxide in the arterial blood (p_aCO_2) is lower than that in the end-expired air (p_ECO_2), with the reverse being true for oxygen, i.e. $p_aO_2 > p_EO_2$ (e.g. Scheid & Piiper, 1972). In the evolved gas exchangers, overlaps in the partial pressure gradients are only achieved in the cross-current system of the lung – air sac system of birds and counter-current system of the fish gills (Maina, 1998, 2000c).

Structure and function of certain gas exchangers

Gills

Water breathing is the inaugural mode of respiration. Gills are gas-permeable evaginated outgrowths from the body surface. They vary greatly in complexity, from the simple external ones of the annelid polychaetes, some nudibranch molluscs, larval teleosts and tadpoles, to the multifunctional internal ones that are contained in branchial chambers, e.g. in fish and crustaceans, or accommodated in a mantle cavity, e.g. in molluscs.

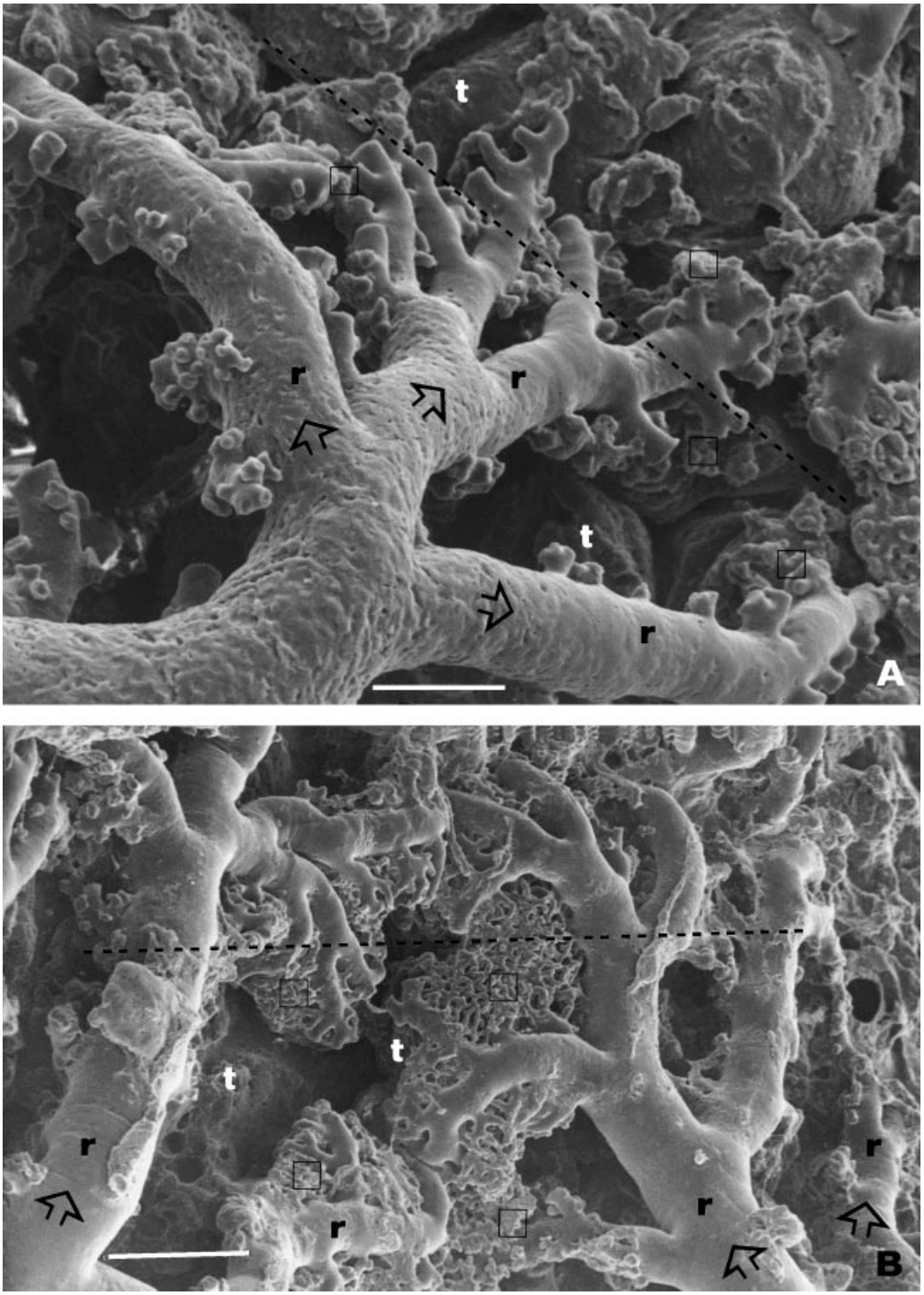

Fig. 6 (A,B) Double latex cast preparation (latex rubber injected into the airways and arteries) of the lung of the domestic fowl, *Gallus gallus* variant *domesticus*, showing the cross-current arrangement between the blood flow (arrows) and the flow of air in the parabronchial lumen (dashed line): venous blood in the intraparabronchial arteries (r) flows in a perpendicular direction in relation to that of the parabronchial air. Also shown is the multicapillary serial arterialization system, i.e. sequential arrangement of blood capillaries (squares) along the lengths of the parabronchi. t, atria arising from the parabronchial lumen. A: scale bar, 25 μm; B: scale bar, 50 μm.

Gills perform functions that include respiration, osmoregulation, acid–base balance, ammonia excretion, regulation of circulating hormones, and detoxification of plasma-borne harmful substances while the simple ones are involved in activities such as locomotion and feeding.

Owing to the multiple roles that they perform, gills must manifest a compromise design. Chloride cells (= ionocytes) (Fig. 7A,B) contain numerous mitochondria and profuse intracytoplasmic tubules (Fig. 7B). Exposed to the surrounding water through pores regulated by pavement cells (Fig. 7A,B), they carry out osmoregulatory function. In teleost gills, secondary lamellae, thin plate-like structures that are located on both sides of the gill filaments (Fig. 1A) and gill filaments of the crustacean gills (Fig. 1B) are the functional respiratory units.

The water–blood barrier of fish gills is thin (e.g. Hughes & Morgan, 1973). Generally, between 1 and 5 μm, thicker in bottom-dwelling fish and elasmobranchs, elaborate epithelium, the primary epithelium, covers the gill filament while a simple one, the secondary epithelium, lines the secondary lamellae. The functions of the gills appear to occur at various sites of the highly differentiated and specialized epithelium. In fish gills, respiratory surface area as well as exposure of the chloride cells to water can be adjusted, thereby optimizing osmoregulation and gas exchange to suit prevalent conditions (e.g. Butler & Metcalfe, 1983).

Pavement cells are simple squamous cells that have microridges (Fig. 7A,B) that are thought to allow extension without mechanical damage (e.g. Knutton et al. 1976). Furthermore, they are associated with roles such as trapping and holding mucous, providing structural integrity to the gill epithelium and increasing the respiratory surface area. Pillar cells bridge the space between the parallel epithelial cells that cover the secondary lamellae. The basement membranes of the pillar cells provide structural integrity, preventing overdistension under undue intramural pressure. The respiratory surface areas of the fish gills correlate with the metabolic demands and the environment in which they live (e.g. Hughes & Morgan, 1973).

Amphibian lungs

Modern amphibians occupy a central position in understanding the fundamental changes that have occurred in the evolution of air breathing. Dual subsistence in water and land has required development of certain respiratory adaptations. The remarkable heterogeneity of the morphology of the amphibian gas exchangers matches that of the diversity of the environments in which the animals live, the lifestyle they pursue, and their pattern of interrupted development.

During the early stages of life, amphibians have transient external and internal gills. Thereafter, lungs and bucco-pharyngeal cavity become highly vascularized, acquiring an important respiratory role. Among air-breathing vertebrates, amphibians have the simplest lungs: rudimentary lungs are adequate in ectothermic, low aerobic metabolism animals (e.g. Guimond & Hutchison, 1976). Though having multiple discretion, at any one moment of their development and for a particular environment, in amphibians, only one gas exchanger may be best refined. Pulmonary vascularization correlates with terrestriality, behaviour and tolerance to dessication. The skin is the main pathway for gas transfer in aquatic species while in terrestrial ones, it has been relegated or rendered redundant. In the lungless salamanders (Plethodontidae), some of which live in cold, well-aerated waters, gas exchange occurs across the skin and buccal cavity (e.g. Gatz et al. 1974).

The caecilians (Apoda) possess long, tubular lungs. In a caecilian species like *Boulengerula taitanus*, the left lung is remarkably reduced or totally missing (e.g. Maina & Maloiy, 1988). However, in aquatic *Typhlonectes compressicauda*, as many as three lungs develop (Toews & MacIntyre, 1978). The lungs of caecilians are internally subdivided, forming air cells that are supported by diametrically placed trabeculae.

In the newts (Urodela), animals that are mostly aquatic, the lungs are poorly vascularized with the internal surface being smooth (e.g. Goniakowska-Witalinska, 1980). The low-metabolism newt, *Triturus alpestris*, has smooth-surfaced lungs with 569 capillary meshes per cm^2 (e.g. Claussen & Hue, 1987). The metabolically active tree-frog, *Hyla arborea*, has more elaborate lungs with 652 blood capillary meshes per cm^2 (Czopek, 1965). Lungs of most amphibians such as *Amphiuma tridactum* and the cane toad, *Bufo marinus*, have an abundance of smooth muscle tissue (e.g. Stark-Vancs et al. 1984), a feature that may explain the high compliance of the lungs (e.g. Hughes & Vergara, 1978). In *Amphiuma*, during expiration, the lung virtually collapses, producing an almost 100% turn-over of inspired air (Stark-Vancs et al. 1984): *Amphiuma* is aquatic but has very well developed lungs.

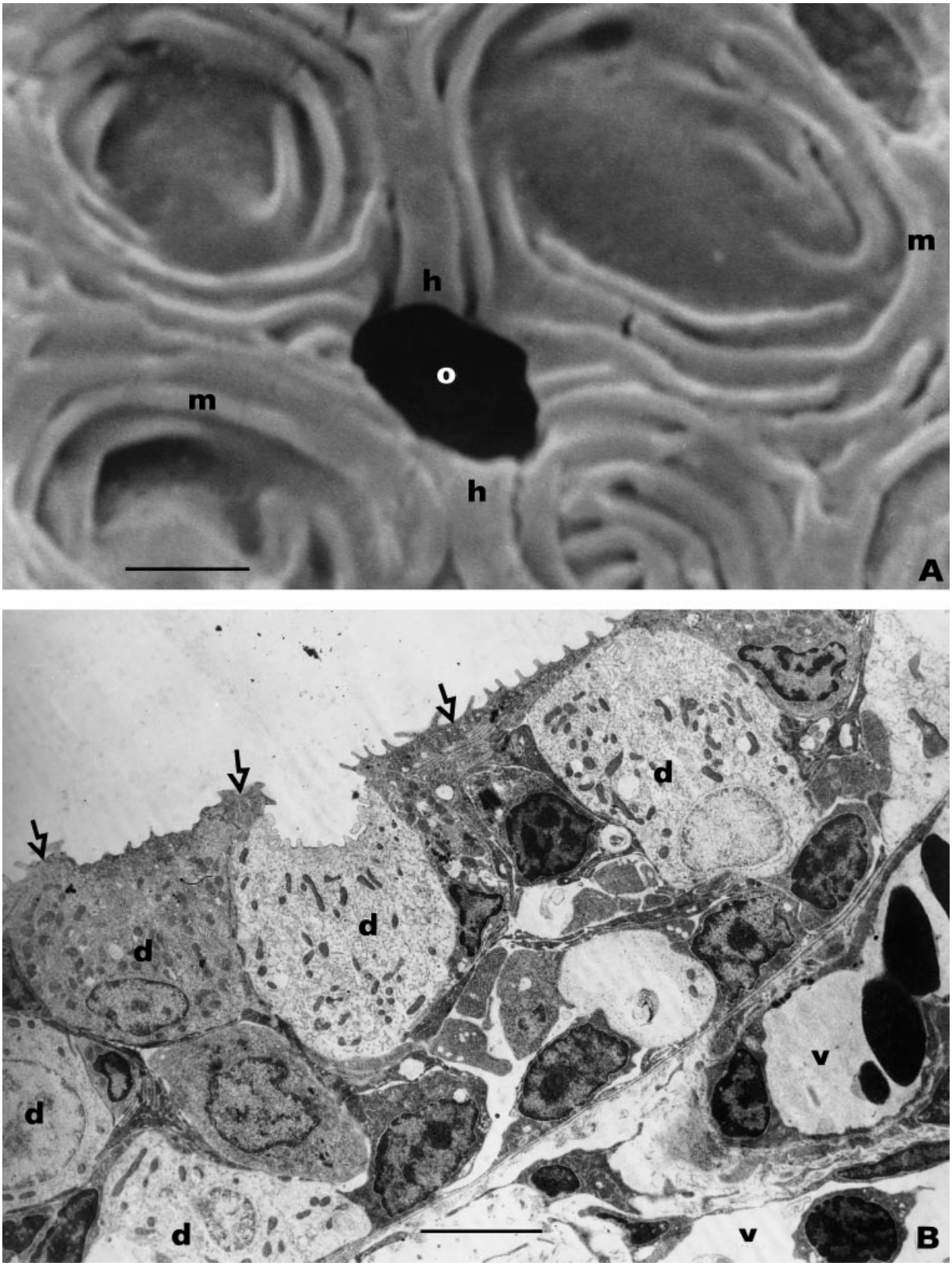

Fig. 7 (A) Surface of the gill filament of *Alcolapia grahami* showing a pore (o) opening onto a chloride cell. h, pavement cells; m, microridges. Scale bar, 5 μm. (B) Chloride cells (d) on the primary epithelium of a gill filament of *Alcolapia grahami*. Arrows, pavement cells with microridges on the outer surface; v, vascular channels. Scale bar, 10 μm.

Morphologically and morphometrically, the lungs of Anura and Apoda are more complex than those of Urodela. On average, the thickness of the blood–gas barrier in the urodeles is 2.59 μm, 2.35 μm in the apodans (caecilians) and 1.89 μm in the anurans (Meban, 1980). The lungs of terrestrial species, e.g. *Bufo marinus* (e.g. Smith & Rapson, 1977), tree frogs, *Hyra arborea* (e.g. Goniakowska-Witalinska, 1986) and *Chiromantis petersi* (Maina, 1989a) are highly elaborate: there are a series of stratified septa that divide the lung into small air cells that range in diameter from 1.45 mm in *Rana pipiens* to 2.3 mm in *Bufo marinus* and *Rana catesbeiana* (Tenney & Tenney, 1970). The aquatic species *Xenopus laevis* has very well developed lungs (Czopek, 1965). Well differentiated pneumocytes as well as dust cells (free phagoctes) have been reported on the surface of some amphibian lungs (e.g. Maina, 1989a).

Reptilian lungs

Reptiles were the first vertebrates that adequately adapted for terrestrial habitation and utilization of lungs as the sole pathways for acquisition of oxygen. They display great pulmonary structural heterogeneity (e.g. Maina et al. 1989b; Perry, 1999): there is no single model reptilian lung. Based on complexity of internal organization, different classifications were suggested by Perry (1989, 1999) – these include:

1 the profusely subdivided (multicameral) ones of turtles, monitor lizard, crocodiles and snakes;

2 the simpler (paucicameral) ones of the chameleons and the iguanids;

3 the saccular, smooth-walled, transparent (unicameral) ones of, for example, the teju lizard, *Tupinambis nigropunctatus*.

The simplest reptilian lungs, e.g. those of Rhynchocephalia, correspond in their organizational complexity to the amphibian lung: a central air duct opens to peripheral ediculae. Arguably, the brochoalveolar lung of mammals and parabronchial lung of birds are thought to have evolved from transformation of a multicameral lung (e.g. George & Shah, 1965; Duncker, 1978; Perry, 1999). In the many highly derived snakes, e.g. Colubridae, Viperidae and Elapidae, the left lung is highly reduced or is totally lacking. Primitive and some general species, e.g. the boas and the pythons, have a left lung (e.g. Luchtel & Kardong, 1981). In Amphisbenia, the right lung is underdeveloped (e.g. Gibe, 1970). The lungs of marine reptilian species have multichambered bronchiolated lungs (e.g. Solomon & Purton, 1984). The elongated lungs of some snakes and amphisbaenids are divided into two functional zones: an anterior respiratory region that is well vascularized and a posterior one that is saccular and avascular (e.g. Stinner, 1982; Maina, 1989b).

The epithelial cells lining the respiratory surface of the reptilian lung are differentiated into type I and II cells (e.g. Daniels et al. 1994). Dust cells (surface macrophages) have been reported in some reptilian lungs, e.g. in the tortoise, *Testudo graeca* (Pastor et al. 1989).

Reptilian lungs have a preponderance of smooth muscle tissue (Maina, 1989b). In the tegu and the monitor lizards, respectively, smooth muscle tissue constitutes 7.4% and 1.3% of the non-trabecular tissue (e.g. Perry et al. 1989). The smooth muscle tissue has been associated with intrapulmonary convective movement of air (e.g. Carrier, 1988). The compliance of the lung of the garter snake, *Thamnophis sirtalis*, is 0.042 mL cm water^{-1} g^{-1}, (Bartlett et al. 1986) 50 times that of a mouse, a mammal of about the same body mass. At the peak of an expiratory phase, the residual volume of air (18 mL kg^{-1}) in the crocodile lung is only 13% of the maximal lung volume (Perry, 1988).

Mammalian (bronchoalveolar) lung

In the mammalian lung, the airway and vascular systems form a complex multigenerational dichotomous branching tree-like arrangement (Maina & van Gils, 2001) (Fig. 8A). For every branching, the airway diameter is reduced by a factor of the cube root of 2 (Weibel, 1984).

Fractal properties in the design of the bronchial, venous and arterial systems of the lung have been described in the mammalian lung (e.g. Weibel, 1991; Maina & van Gils, 2001). Morphogenetically, mammalian lungs are constructed by 'moulding' three fractal conduits, namely arterial, venous and bronchoalveolar systems (Fig. 8A). Fractal dimensions of the diameter elements of the arterial and the venous tree in the human lung are 2.71 and 2.64, respectively, while the equivalent values for the length element are 2.97 and 2.86, respectively (e.g. Huang et al. 1996). Briggs (1992) observed that the pulmonary vascular system has an effective fractal dimension of 3, with the arteries alone having that of 2.7. High fractal dimensions maximize respiratory surface area and allow perfusion at low

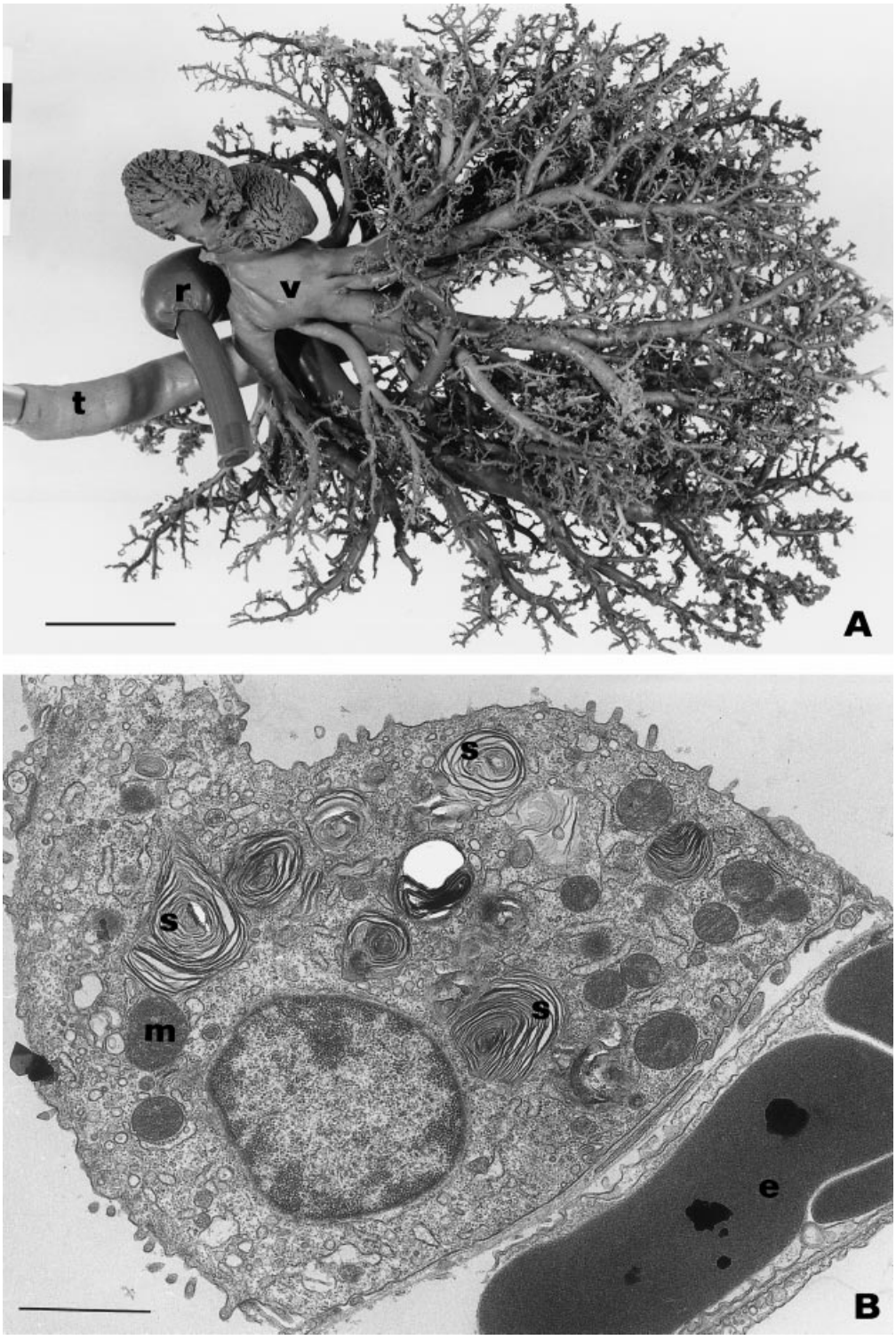

Fig. 8 (A) Ventral view of the airway system arising from the trachea (t), the pulmonary arterial system arising from the pulmonary artery (r), and the pulmonary venous system arising from the pulmonary vein (v) of the pig lung, *Sus scrofa*. The alveolar and blood capillary meshwork was physically pruned to reveal the branching patterns and the fractal nature of the three conducting systems. Scale bar, 1 cm. (B) Type II cell of the lung of a bat *Miniopterus minor* showing osmiophilic lamellated bodies (s), the precursors of surfactant. e, erythrocyte; m, mitochondria. Scale bar, 10 μm.

energy cost. The human lung is ventilated every day with about 12 000 L of air and perfused by half as much blood (e.g. Burri, 1985).

Transported by bulk-flow (convention) in the initial (large) parts of the bronchial system and mainly by diffusion in the terminal (fine) sections of the airway system, the inspired air ultimately reaches the alveoli where it is exposed to capillary blood across a thin, extensive tissue barrier. Measured in milliseconds, the duration when air and blood are exposed to each other is fleetingly brief (e.g. Lindstedt, 1984). Alveoli, rather polymorphic air spaces that are delimited by well vascularized septa (Fig. 2A,B), measure about 50 μm in diameter in shrews and bats and as much as 1 mm in the placid sirenians (dugongs and manatees) (Tenney & Remmers, 1963). The alveolar surface is mainly lined by type I (Fig. 4A) and type II cells (Fig. 8B): type II cells secrete surfactant. In the human lung, there are 23×10^6 pulmonary pneumocytes with the type I cells constituting 8% of the total number (Crapo et al. 1983) but covering about 90% of the alveolar surface.

Avian (parabronchial) lung

The avian respiratory system, the lung – air sac system, is the most complex and efficient gas exchanger that has evolved in air-breathing vertebrates. The compact and virtually constant-volume avian lung (Fig. 3A) has been totally uncoupled from the compliant, avascular air sacs. During respiration, the volume of the lungs changes by a mere 1.4% (Jones et al. 1985). Amazingly, compression of the lung does not lead to significant collapse of the air capillaries (Macklem et al. 1979). Though birds have smaller lungs per unit body mass than mammals by a factor of 27% (e.g. Maina et al. 1989a), the rigidity of the avian lung has permitted substantial increase in the respiratory surface area by intense internal subdivision.

The capacity of supplying large amounts of oxygen by the avian lung, e.g. for flight at high altitude, is exceptional by mammalian standards. A bird in flight uses oxygen at 2.5 times that of a mammal running fast on the ground (e.g. Thomas, 1987). The respiratory efficiency of the avian lung ensues from complex synergy of structural and functional parameters. The main properties that impart high respiratory efficiency on the lung – air sac system of birds are:

1 cross-current design and inbuilt multicapillary serial arterialization system;
2 auxilliary counter-current system;
3 large tidal volume;
4 large cardiac output;
5 continuous and unidirectional parabronchial ventilation (in the palaeopulmo);
6 short pulmonary circulatory time;
7 superior morphometric parameters.

The total volume of blood in the avian lung constitutes as much as 36% of the lung volume, with 58–80% of it in the blood capillaries (e.g. Duncker & Güntert, 1985). The pulmonary capillary blood volume in birds is 2.5–3 times greater than in the mammalian lung where only 20% of it is found in the alveolar capillaries (Weibel, 1963).

The avian lung respiratory system has great functional reserve. This is shown by the following findings:
1 cold exposure does not cause respiratory distress (e.g. Koteja, 1986);
2 isolation of the thoracic and abdominal air sacs, a surgical procedure that renders about 70% of the total air capacity non-functional, does not adversely affect gas exchange efficiency (e.g. Brackenbury & Amaku, 1990);
3 during flight, at ambient temperatures between 12 and 22 °C, lung oxygen extraction does not change (e.g. Torre-Bueno, 1978);
4 in the bar-headed goose, *Anser indicus*, neither ventilation nor pulmonary gas transfer is limiting in a bird exercising in a hypoxic environment (7% oxygen) (e.g. Fedde et al. 1989).

The lungs of small and metabolically active species of birds show distinct morphometric refinements (e.g. Maina et al. 1989a). The lungs of small passerine species that have a higher metabolic rate and operate at a higher body temperature are superior to those of non-passerines (Maina, 1984). Gliding and soaring birds that expend less energy in flight have relatively inferior lungs (Maina, 1987b). Galliform birds, e.g. the domestic fowl, *Gallus gallus* variant *domesticus*, a bird that has been domesticated for over 5000 years (West & Zhou, 1988), the guinea fowl, *Numida meleagris*, and the turkey, *Melleagris gallopavo*, have low pulmonary diffusing capacities (e.g. Abdalla & Maina, 1981; Abdalla et al. 1982; Timmwood et al. 1987). The lowest pulmonary morphometric diffusing capacities have been reported in the emu, *Dromiceius novaehollandiae* (Maina & King, 1989), a large flightless bird that has evolved in a habitat with few predators. The humboldt penguin, *Spheniscus humboldti*, has a remarkably thick blood–gas barrier (0.530 μm) (Maina & King, 1987) that

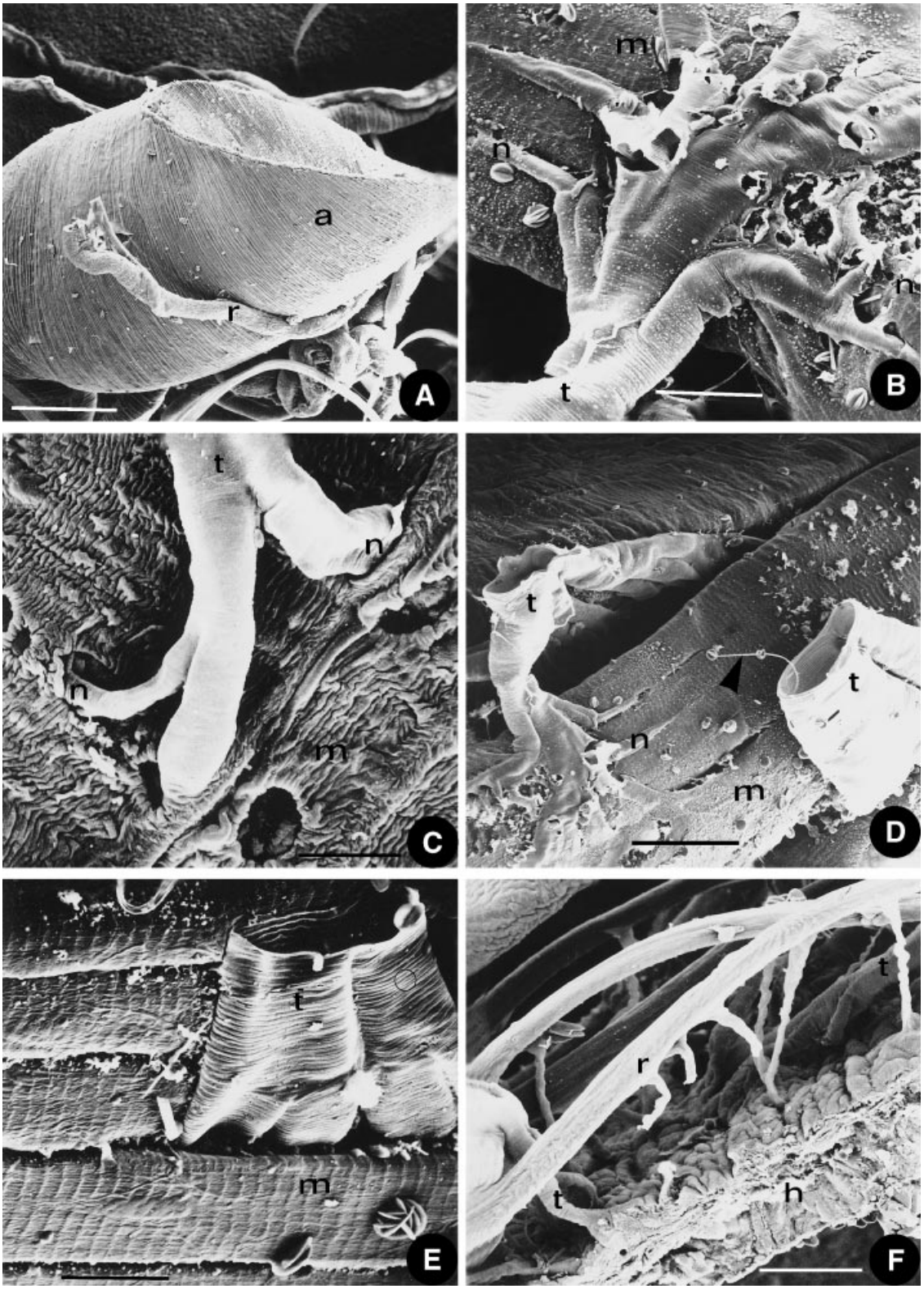

Fig. 9 Insect respiratory system: air sacs and tracheal system. (A) Air sac (a). r, Malphigian tubule. Scale bar, 0.40 mm. (B–E) Tracheal air supply to the flight muscles (m). t, trachea; n, tracheole; ¡, taenidia; ►, taenidium. B: scale bar, 20 μm; C: scale bar, 15 μm; D: scale bar, 82 μm; E: 100 μm. (F) Tracheal air supply (t) to the wall of the gastrointestinal system (h). r, Malpighian tubule. Scale bar, 90 μm. Preparations A, B, D, E, F are from a grasshopper, *Chrotogonus senegalensis*, and C is from a locust, *Schistocerca gregaria*.

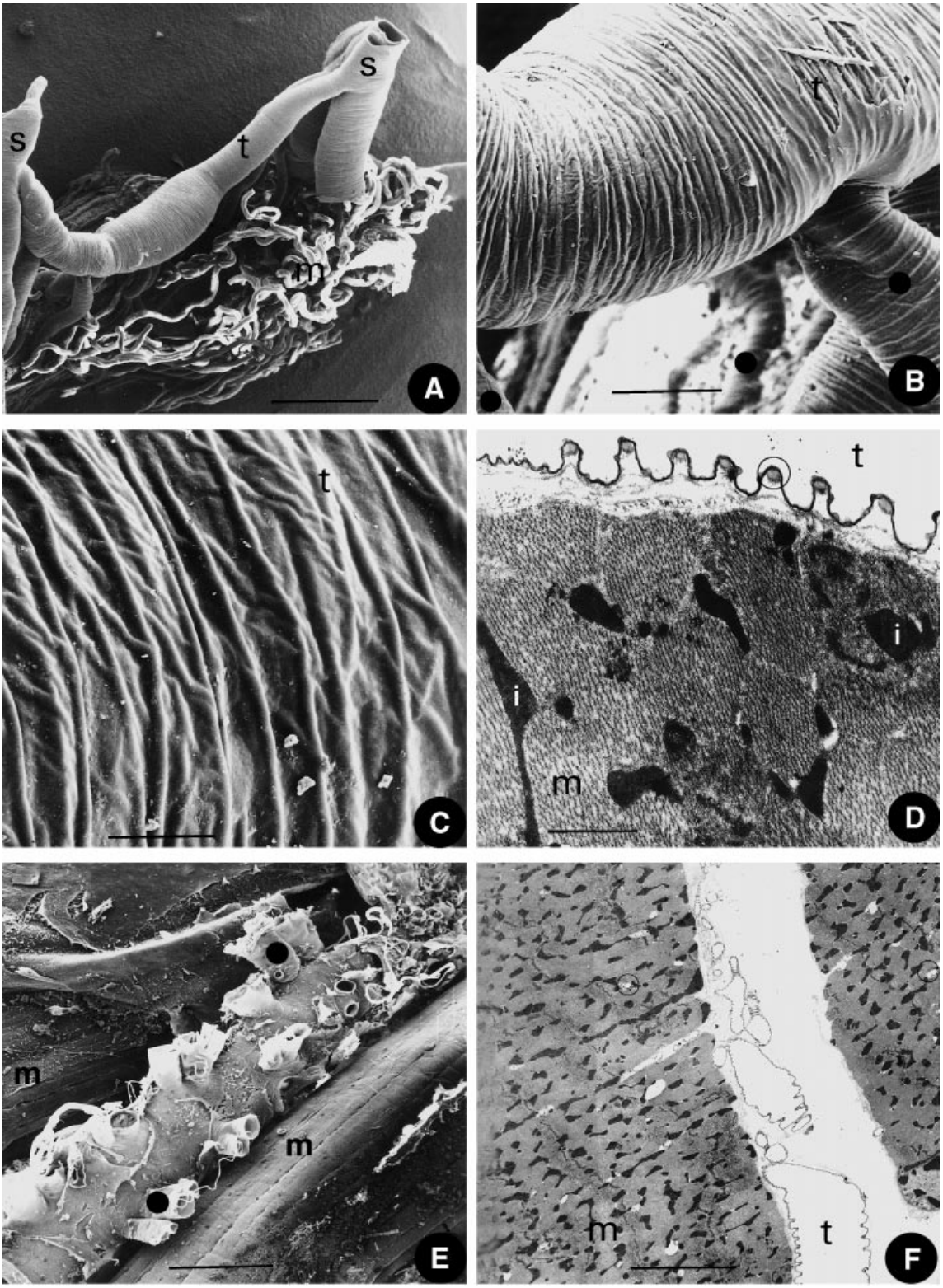

Fig. 10 (A) A transverse trachea (t) connecting two tracheal trucks emerging from spiracles (s). m, Malphigian tubules. Scale bar, 1.3 mm. (B) Transverse trachea showing the spiral taenidia (t) that support it. ●, tracheal branches. Scale bar, 0.14 mm. (C) Surface of a trachea showing ridges formed by taenidia (t). Scale bar, 0.03 mm. (D) Tracheole (t) running through a flight muscle (m). i, mitochondria. Scale bar, 0.53 μm. (E) A large trachea running through a flight muscle (m) giving rise to branches (●) that invade the muscle tissue. Scale bar, 0.75 mm. (F) Trachea (t) lying on a flight muscle (m). ○, tracheoles within muscle tissue. Scale bar, 0.5 μm. All figures from a grasshopper, *Chrotogonus senegalensis*.

conceivably enables the lung to resist collapse from hydrostatic pressures in an excellent diver (Butler & Woakes, 1985).

Regarding structure and function, the analogy between the lung – air sac system of birds and the tracheal system of insects [gas exchangers are ventilated continuously and unidirectionally and air sacs (Fig. 9A) increase the tidal volume], animals separated by about 200 million years of evolution, is a classical case of analogous design imposed by flight, an energetically highly expensive mode of locomotion.

Insectan tracheal system

Tracheal respiration is unique for its remarkable efficiency and simplicity of design. With the assistance of air sacs (Fig. 9A), oxygen is delivered directly to the tissue cells by rigid trachea and tracheoles (Figs 9B–F and 10A–F), with minimal drop in partial pressure. In adult bug *Aphelocheirus*, e.g. between the spiracles and the tracheoles, the pO_2 drops by only 0.3 kPa (Thorpe & Crisp, 1941). The respiratory and circulatory systems have been totally disengaged, relegating the latter from meaningful role in gas exchange. With spiracles serving as a carburettor, in mechanical terms, the tracheal system simultaneously serves as a compressor and an exhaust pipe. The air sacs and the trachea are supported by circular or helical cuticular taenidia (Figs 9A,B,D,E and 10A–D) (e.g. Maina, 1989c). Functionally, the insectan tracheal system resembles plant leaves, where oxygen is delivered directly to the cells without passing through a circulatory system: the spiracles compare to stomatas.

Every cell in the insectan body is directly or indirectly supplied with oxygen. The tracheoles (e.g. Fig. 9B,D,F), the finest branches of the tracheal system, are analogous to the vertebrate blood capillaries. They can supply 10 times more oxygen per gram of tissue than the blood capillaries (Steen, 1971). In highly metabolically active tissues, tracheoles may be as narrow as 0.2 μm in diameter and may indent cells in a manner of a finger poked into a balloon (Wigglesworth, 1972). Mitochondria cluster round tracheoles forming what Edwards et al. (1958) termed 'mitochondrial continuum'. Air sacs increase tidal volume by as much as 70% of the total air capacity produced by trachea alone and reduce the longitudinal diffusion gradient for oxygen along the gas exchange pathway. The metabolic rates of the flight muscles of insects are some of the highest reported for any tissue (e.g. Kammer & Heinrich, 1978), with values that approach those of pure microbial cultures (e.g. Hughes & Wimpenney, 1969). In much the same way as the degree of capillarization of the tissues correlates strongly with metabolic activity in vertebrates, in insects, tracheolar density is dependent on the metabolic activities and the pO_2 in particular organs and parts of the body (e.g. Steen, 1971).

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