OSTRICH 2011, 82(2): 115–127
Printed in South Africa — All rights reserved

OSTRICH
ISSN 0030–6525 EISSN 1727–947X
doi: 10.2989/00306525.2011.603465

A study of gross morphological and histological syringeal features of true francolins (Galliformes: *Francolinus*, *Scleroptila*, *Peliperdix* and *Dendroperdix* spp.) and spurfowls (*Pternistis* spp.) in a phylogenetic context

Tshifhiwa G Mandiwana-Neudani[1,2]*, Cecilia Kopuchian[3], Graham Louw[4] and Timothy M Crowe[2]

[1] *Department of Botany, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa*
[2] *DST/NRF Centre of Excellence at the Percy FitzPatrick Institute of African Ornithology, Private Bag X3, Rondebosch 7701, South Africa*
[3] *División Ornitología, Museo Argentino de Ciencias Naturales "B. Rivadavia" Av. Ángel Gallardo 470 (C1405DJR), Buenos Aires, Argentina*
[4] *Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Anzio Rd, Observatory 7925, South Africa*
* *Corresponding author, e-mail: timothy.crowe@uct.ac.za*

Modern taxonomies of francolins recognise 41 congeneric species, forming the largest genus of terrestrial gamebirds (Galliformes). Recent molecular, ecological and behavioural studies challenge this view, suggesting that they comprise two unrelated, monophyletic groups. There are 'true' francolins (*Francolinus*, *Dendroperdix*, *Peliperdix* and *Scleroptila* spp.) that are relatively small, ground-roosting birds, and spurfowls (*Pternistis* spp.) that are large birds that can roost in trees. This study explores gross morphological and histological syringeal anatomy of francolins, spurfowls and sister taxa to test whether differences are concordant with a molecular-based hypothesis. Differences found were the presence of a shield- versus diamond-shaped tympanum among francolins and spurfowls respectively. The first bronchial half rings are mineralised among francolins except in *Dendroperdix sephaena*, whereas almost no mineral deposition was observed among spurfowls. Histologically, francolins have a small, rounded pessulus (except in *D. sephaena*, which has a rounded, larger pessulus) contrary to the larger pessulus observed among spurfowls, which is rounded and triangular in *Pternistis capensis* and *P. natalensis*. Both gross and histological similarities within, and differences between, francolin and spurfowl syringes support this division. However, *D. sephaena* shows intermediate features between francolins and spurfowls.

Introduction

Syringeal characters in taxonomy and systematic studies

The syrinx (the avian voice box) is generally located in the base of the neck, at the junction of the trachea and bronchi (Ames 1971, Lewis 1983, Seller 1983, Gill 1990). It can be formed from either the tracheal or bronchial tissues or both. Myers (1917) categorised three types of syrinx based on its location relative to the trachea and the bronchi: 'syrinx trachealis' if it is found at the lower end of the trachea, 'syrinx bronchialis' in the case where the syrinx is located below the bifurcation, and 'syrinx tracheo-bronchialis' when the syrinx is located at a position that includes both the lower end of the trachea and the upper parts of the bronchi.

The structure of the syringes of song birds has been widely compared with that of the non-song birds (Frank et al. 2006), with passerine birds known to produce complex vocalisations as opposed to the relatively simple vocalisations given by many non-passerine birds. Whether the complexity of the vocalisations is dependent on the number or the complexity of the syringeal components is a question that requires detailed investigation into the function of each part. This is because of the fact that certain non-passerine species, e.g. cockatiels *Nymphicus hollandicus*, which are known to have just three pairs of syringeal muscles and two pairs of tracheal muscles (Larsen and Goller 2002), can mimic many types of sound (Tsukahara et al. 2008). The presence or absence of certain components of the syringes or their musculature contribute largely to voice production, and has been found to play a significant role in the classification of birds. This is supported by evidence in studies of the syringes of the Domestic Chicken *Gallus domesticus*, the male Mallard *Anas platyrhynchos* and the Greater Sage-Grouse *Centrocercus urophasianus* (Myers 1917, Frank et al. 2006, Krakauer et al. 2009, respectively).

The syrinx is anatomically complex and interspecifically diverse even in species that lack special structures. Syringeal morphology has been studied repeatedly and found to be informative in many systematic studies on Passeriformes, e.g. Tyrannidae (Lanyon 1986, Prum and Lanyon 1989, Mobley and Prum 1995), Pipridae (Prum 1992) and Furnariidae (Zimmer et al. 2008), and non-passerines, e.g. Anatidae (Delacour and Mayr 1945, Humphrey 1955, 1958, Johnsgard 1961, Livezey 1986), Charadriiformes (Brown and Ward 1990), Falconidae (Griffiths 1994a, 1994b) and Psitacidae (Gaban-Lima and Höfling 2006). As an example of the utility of syringeal characters in non-passerines, Livezey (1986) in his phylogenetic analysis of Anseriformes studied tracheal characters and found them to be synapomorphies or autapomorphies in some clades.

Because of this known utility of syringeal characters in studies of phylogenetic relationships among non-passeriformes taxa and in the spirit of using multiple lines of evidence to test hypotheses of monophyly (Templeton 1989, Farais et al. 2000, Pruett and Winker 2010), this study explored the gross anatomy and histology of francolin and spurfowl syringes and the concordance of this feature with phylogenetic relationships supported in previous studies on this group (Crowe and Crowe 1985, Milstein and Wolff 1987, Crowe et al. 1992, Bloomer and Crowe 1998, Crowe et al. 2006).

This is, to our knowledge, the first truly comparative paper of syringeal morphology in francolins and spurfowls. Also, we here present data on a number of lesser-known species in a group that tends to be dominated by data from a few domesticated or managed north-temperate species.

Taxonomy, distribution, ecology and morphology

Based on traditional morphological research, the 41 currently recognised species of francolins were placed within a single genus (*Francolinus*) within the order Galliformes and family Phasianidae (Hall 1963). They are distributed throughout sub-Saharan Africa (with an isolated set of populations of one species in Morocco), the Middle East and Asia (Johnsgard 1988, Madge and McGowan 2002), and are adapted to a variety of habitats, comprised of primarily tropical and unforested vegetation types (McGowan 1994). All francolins have 14 tail feathers and most species are sexually monomorphic, with males having single- or double-spurred tarsi (Hall 1963, Johnsgard 1988).

However, relatively recent morphological, ecoethological and molecular studies of francolins (Crowe and Crowe 1985, Milstein and Wolff 1987, Crowe et al. 1992, Bloomer and Crowe 1998, Crowe et al. 2006) have suggested that they form two distantly related lineages: 'true' francolins and spurfowls (Figure 1). 'True' francolins (allocated to four genera: *Francolinus*, *Dendroperdix*, *Peliperdix* and *Scleroptila*) are relatively small, ground-roosting birds with striped and barred rufous dorsal plumage resembling that of quails *Coturnix* spp., with only males possessing relatively small tarsal spurs. Spurfowls are placed into one genus (*Pternistis*) and are generally larger, can roost in trees, and have dark dorsal plumage usually vermiculated with white or buff, and both sexes usually have spurred (often doubly) tarsi that are much longer in males. Furthermore, spurfowls generally emit atonal, raucous, grating advertisement calls (given at dawn and dusk), whereas francolins have more tonal, often whistling, calls (Milstein and Wolff 1987). This distinction between the two assemblages becomes blurred owing to the Crested Francolin *Dendroperdix sephaena* which, like other francolins, has quail-like plumage but, like spurfowls, it has long tarsal spurs, roosts in trees and has an advertisement call with both grating and tonal elements (Milstein and Wolff 1987). It was on the basis of this 'linking form' that Hall (1963) decided to place all 41 species into a single genus, *Francolinus*. The decisive evidence against this placement came from DNA-based phylogenetic studies (Bloomer and Crowe 1998, Crowe et al. 2006), which place true francolins with jungle fowls (*Gallus* spp.) and bamboo partridges (*Bambusicola* spp.), and spurfowls with Old World quails (*Coturnix* spp.) and some Old World partridges (*Alectoris*, *Perdicula*, *Tetraogallus* and *Ammoperdix* spp.) (Figure 1).

In this context, the aim of this study was to examine the gross morphological and histological anatomical structure of the syringes of selected francolins, spurfowls and their putative sister taxa, as well as to determine whether there are any syringeal characters that could be taxonomically and phylogenetically informative in investigating the diphyletic status of the 'true' francolins and spurfowls.

Materials and methods

Syringeal sampling (gross morphology and anatomy)

We examined syringes from species that are representative of francolins and spurfowls and those that are from their sister taxa (Table 1): Common Quail *Coturnix coturnix* and Chukar Partridge *Alectoris chukar* (sisters of spurfowls), *G. domesticus* (sister to francolins) and Helmeted Guineafowl *Numida meleagris* (a distant relative to chickens, quails, partridges, francolins and spurfowls).

It is appropriate at this point to highlight some of the differences between the syringes of male and female birds. As explained by Frank et al. (2007), the syrinx of male and female birds may have morphological differences that in turn could determine the properties of voices of birds. However, Appel (1929) did not find differences between the syringes of males and females in his research work in the Brown Leghorn Fowl *Gallus gallus*, a galliform he studied intensively, even in ovariectomised females.

On the other hand, we avoided examining syringes from immature birds in order to ensure that differences found between syringes were not because of age differences between individuals. As Hogg (1982) described in Galliformes, mineralisation begins well before maturity is reached and virtually achieved its final extent during the growing period, so all adult birds are considered to have a fully developed syrinx.

Of course, it would be ideal to confirm our observations by analysing more individuals of each of the species in this study. However, given the difficulty experienced in gathering multiple syringeal samples (for male and female individuals of a particular species), and the inability to determine the sex of some specimens from which syringes were extracted, we analysed what we managed to acquire believing in the value of these results.

Analyses

All syringes examined for gross morphological and anatomical purposes were dissected from frozen whole bird specimens with the exception of three that were dissected from alcohol-preserved whole specimens provided by the National Museum of Natural History (Ditsong Museums of South Africa), formerly Transvaal Museum (Northern Flagship Institution). The syringes were immersed in 70% ethanol until such time that they were processed.

Syrynx gross morphology

We followed the double-staining protocol in Cannell (1988) for the clearing and staining procedures of the syringes. Ossified tissues stained red with Alizarin red, cartilaginous

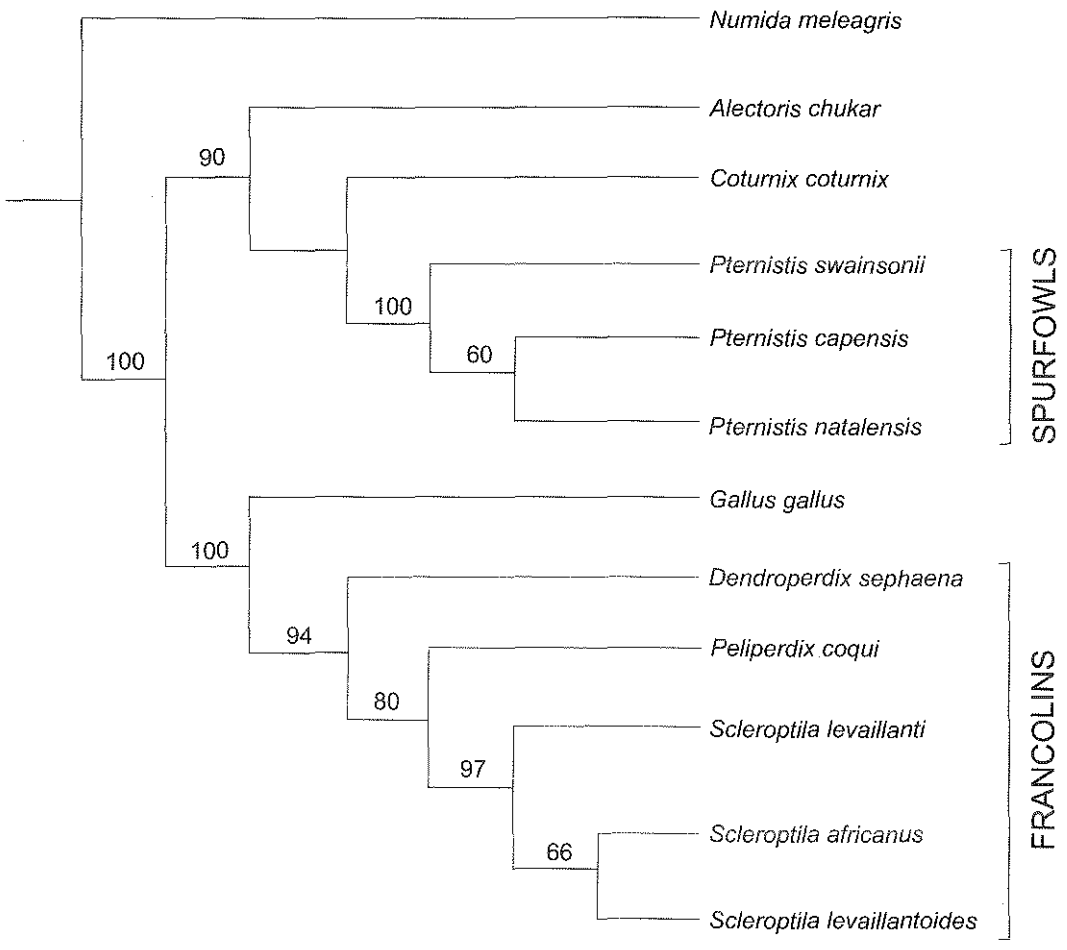

Figure 1: Strict consensus cladogram for some 'true' francolins (*Dendroperdix*, *Peliperdix* and *Scleroptila* spp.) and spurfowls (*Pternistis* spp.) modified from Crowe et al. (2006). Numbers at nodes are jackknife support values

Table 1: List of species for which syringes were analysed

Common name	Scientific name	Gross morphology			Histology		
		Sample name[1]	Sex[2]	Age	Sample name[1]	Sex[2]	Age
Grey-winged Francolin	*Scleroptila afra*	TMC67	M	Adult	TMC01	F	Adult
Orange River Francolin	*S. levaillantoides*	TMC65	F	Adult			
Red-winged Francolin	*S. levaillantii*	TMC60	F	Adult	TMC15	F	Adult
Crested Francolin	*Dendroperdix sephaena*	TM78245	M	Adult	TMC13	M	Adult
Coqui Francolin	*Peliperdix coqui*	TM75627	F	Adult			
Cape Spurfowl	*Pternistis capensis*	TMC70	M	Adult	TMC07	M	Adult
Swainson's Spurfowl	*P. swainsonii*	TMC48	M	Adult			
Natal Spurfowl	*P. natalensis*	TM60042	?	Adult	TMC20	M	Adult
Common Quail	*Coturnix coturnix*	TMC50	F	Adult	TMC14	F	Adult
Chukar Partridge	*Alectoris chukar*	TM74489	?	?			
Helmeted Guineafowl	*Numida meleagris*	TMC45	M	Adult	TMC17	F	Adult
Domestic Chicken	*Gallus domesticus*	TMC30	?	Adult	TMC02	?	Adult

[1] TM, Transvaal Museum; TMC, Timothy M Crowe (Percy FitzPatrick Institute of African Ornithology, University of Cape Town)
[2] ? = Unknown

tissues stained blue with Alcian blue and muscles stained brownish in Lugol solution. Cleared and stained syringes were examined using a Leica S8APO stereomicroscope and LAS EZ version 1.7.0 software. Syringes of large birds such as *Gallus gallus*, *Numida meleagris*, *Pternistis capensis*, *P. swainsonii* and *P. natalensis* were stained for a few more days compared to the francolins owing to the difference in size.

Histology

For the tissue-sectioning procedure, the syringes were transferred from 70% ethanol to 10% buffered formalin to stay overnight and then taken through a series of 70%, 90% and absolute ethanol and cleared in xylene (1 h in each solution). They were then embedded in paraffin wax and longitudinal sections of 5 µm thickness were cut. Two stains were used, namely the haematoxylin and eosin (Bancroft and Gamble 2002) stain for more general biological examination and a more specialised multiple stain, orcein-picroindigocarmine (Steven et al. 2000). The orcein-picroindigocarmine stain was appropriate in analysing bird syringes since it has the potential to differentiate tissues and their components.

Tissue sections were stained with orcein, indigocarmine and picric acid following the Steven et al. (2000) protocol. The sections were deparaffinised and taken to distilled water, stained with orcein solution for 45 min at room temperature and rinsed in distilled water. Sections were then differentiated in 96% ethyl alcohol (two times) for 30 s each, rinsed in distilled water and stained with picroindigocarmine solution for 30 min. Slides were drained and differentiated in 70% ethyl alcohol for 2 min and finally mounted with Entellan and examined using a Nikon Stereoscopic Zoom microscope (NIS-Elements version 2.10). The sectioning of tissues was more difficult for the larger birds examined and this was a contributing factor in the distortion of those sections. Finally, even though the staining of whole syringes and the sectioning of samples of larger syringes was challenging, most of the stained syringes and sections were intact and clear with the exceptions of Figures 5d, 6d and 6f only.

Results

Syringeal gross morphology

The basic structure of the syringeal morphology (Figure 2) of a typical francolin/spurfowl is characterised by cartilaginous rings (which stained blue with Alcian blue), a very distinctive mineralised tympanum (which stained red with Alizarin red) and muscles (which stained brownish in Lugol solution). All the species analysed have a typical 'tracheobronchialis' type of syrinx (Figures 3, 4 and 5), which means that both the tracheal and the bronchial tissues are involved in the formation of the syrinx and, possibly, in shaping the structure of their vocalisations.

Two significant gross morphological differences between true francolins and spurfowls were observed. Firstly, unlike their putative sister taxon *G. domesticus*, which has a mineralised triangular-shaped tympanum (Figure 4a), francolins (Figure 4b–f) and *N. meleagris* (Figure 3) have a shield-like calcified tympanum clearly visible from the ventral perspective (Table 2). *Numida meleagris*, *G. domesticus* and francolins show extensive mineral deposition in the tympanum in both ventral and dorsal views but Coqui Francolin *Peliperdix coqui* and Red-winged Francolin *Scleroptila levaillantii* (Figure 4c and d, respectively) show reduced mineral deposition on the dorsal side compared with the ventral side. In spurfowls, as in their putative sister taxa *A. chukar* and *C. coturnix* (Figure 5a and b, respectively), the tympanum is diamond-shaped with the degree of calcification not as extensive as in francolins and relatives (Figure 5c–e). Secondly, on the ventral side, the first bronchial half rings are well mineralised in *G. domesticus* (Figure 4a) and francolins (Figure 4c, d and f) but not so in *D. sephaena* (Figure 4b), spurfowls (Figure 5c–e) and their relatives *A. chukar* and *C. coturnix* (Figure 5a and b).

Another observation was that some species such as *S. levaillantoides*, *G. domesticus* and *N. meleagris* show mineral deposition in some tracheal rings.

Histology

The histological structure of the syringes of francolins, spurfowls and their near relatives appears to be basically similar but with some differences observed (Figure 6). The pessulus, which is present in all the species examined (Figure 6a–h), varies considerably in size and shape. It is generally small and rounded in the two true francolins, i.e. *S. levaillantii* and Grey-winged Francolin *S. afra*, and *N. meleagris* (Figure 6a, b and h, respectively) (Table 3). The pessulus is large and also rounded in *D. sephaena* (Figure 6c) and in spurfowls even though it is almost triangular in Natal Spurfowl *Pternistis natalensis* (Figure 6f) and *C. coturnix* (Figure 6g). It is markedly large and triangular in *G. domesticus* (Figure 6d). Myers (1917) made similar observations in *G. domesticus*.

A distinctive interclavicular air sac, which is bound by the internal tympaniform membrane, is found in the two francolins (*S. levaillantii* and *S. afra)*, Cape Spurfowl *Pternistis capensis*, *C. coturnix* and *N. meleagris* (Figure 6a, b, e, g and h, respectively) and is absent in *D. sephaena* and *G. domesticus* (Figure 6c and d, respectively), while its presence could not be determined in *P. natalensis* (Figure 6f). It was observed that the inner wall of the interclavicular air sac and sometimes the medial bronchial wall are lined with a layer of connective tissue that differs in thickness. Connective tissue fills spaces and provides support to organs. The francolins *S. levaillantii* and *S. afra* (Figure 6a and b) and their relative *G. domesticus* (Figure 6d) have thin connective tissue lining the walls of the membranes that tends to be moderate in *P. natalensis*, *C. coturnix* and *N. meleagris* (Figure 6f, g and h, respectively). *Numida meleagris* has connective lining that runs along the medial bronchial walls. *Pternistis capensis* (Figure 6e), as in *D. sephaena* (Figure 6c), has a large amount of connective tissue that is restricted to the far corners of the internal tympaniform membrane. This tissue pushes against the internal tympaniform membrane forcing it to expand and hence results in the narrowing of the surrounding airway. On the other hand, the external tympaniform membrane, which is found in all the species examined, differs remarkably in length. This structure is extremely long in *G. domesticus* (Figure 6d) and much shorter in the other species (Figure 6a–c and e–h).

Discussion

Although limited samples and characteristic features were covered in this study, the syringeal structure of francolins and spurfowls is basically the same. However, there are some marked differences that distinguish francolins from spurfowls. The syrinx gross morphology is generally consistent and is in support of the split of francolins versus

spurfowls. The shape of the tympanum places *D. sephaena* decisively with other francolins even though its first bronchial half rings are not mineralised as in other francolins and their relative *G. domesticus*. However, *D. sephaena*, as in the two spurfowls *P. capensis* and *P. natalensis* and their evolutionary relatives *C. coturnix* and *A. chukar*, shows no mineral deposition in their first bronchial half rings with *P. swainsonii* showing short first bronchial half rings with very little mineralisation. Thus, the aspect of mineral deposition is not fully consistent among francolins and spurfowls. Our observation on the degree of mineralisation in tracheal rings of *S. levaillantoides*, *G. domesticus* and *N. meleagris*

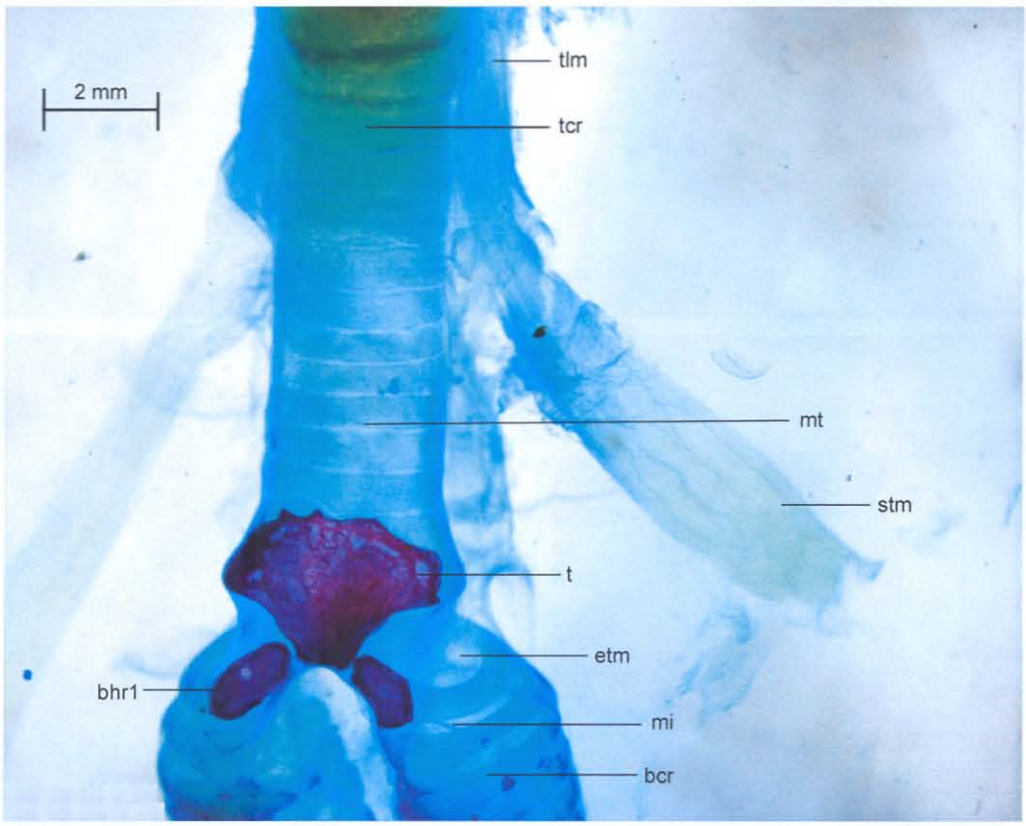

Figure 2: Example of a ventral view of the syrinx of a typical Coqui Francolin *Peliperdix coqui* with illustration of the different parts. tlm = Tracheo-lateralis muscle, tcr = tracheal cartilaginous ring, mt = membrana-trachealis, stm = sterno-trachealis muscle, t = tympanum, etm = external tympaniform membrane, bhr1 = bronchial half ring 1, mi = membrane-interanularis, bcr = bronchus cartilaginous ring. Red colour = mineralised cartilage, blue = cartilage, transparent light brown = muscle

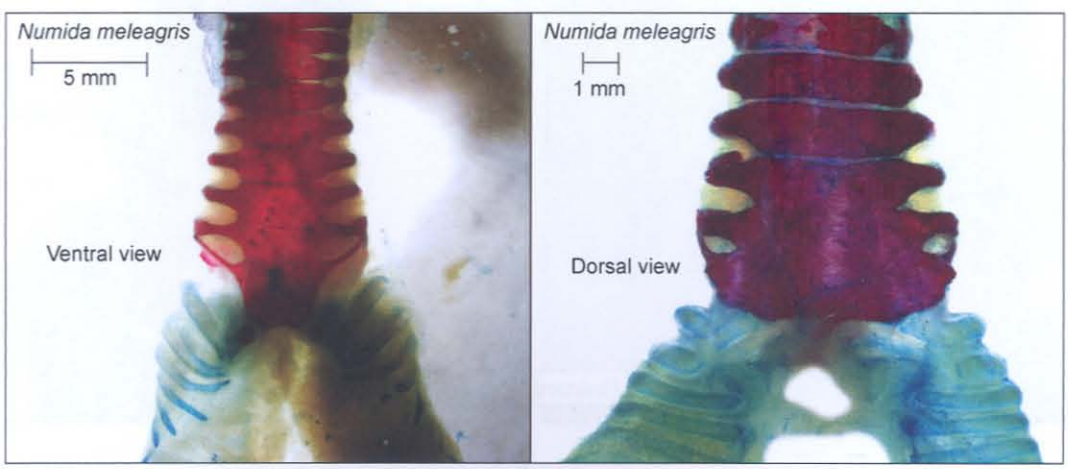

Figure 3: Ventral (left) and dorsal (right) views of the syrinx of Helmeted Guineafowl *Numida meleagris*. Red colour = mineralised cartilage, blue = cartilage, transparent light brown = muscle

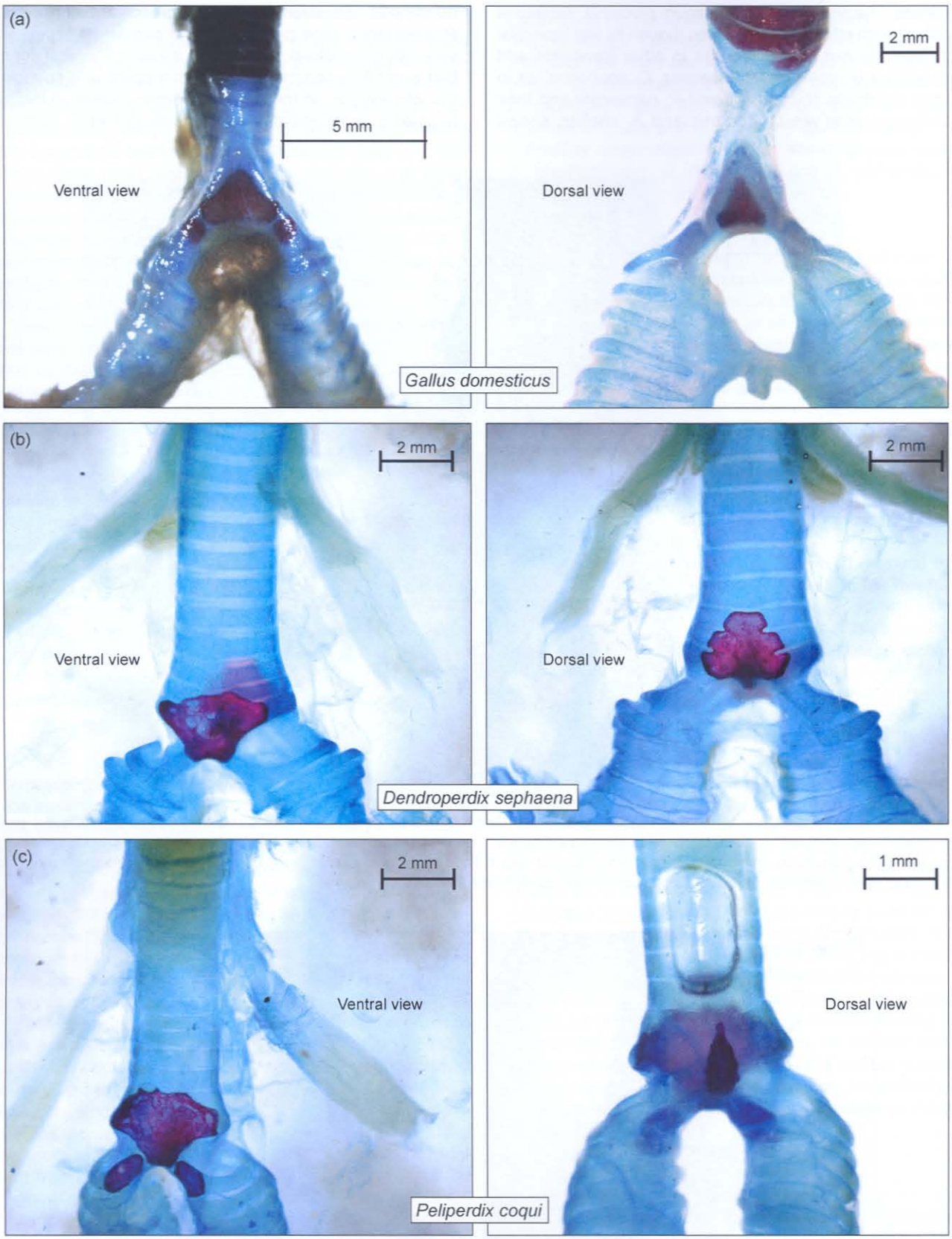

Figure 4: Ventral (left) and dorsal (right) views of the syringes of (a) *Gallus domesticus*, (b) *Dendroperdix sephaena*, (c) *Peliperdix coqui*, (d) *Scleroptila levaillantii*, (e) Orange River Francolin *S. levaillantoides* and (f) *S. afra*. Red colour = mineralised cartilage, blue = cartilage, transparent light brown = muscle

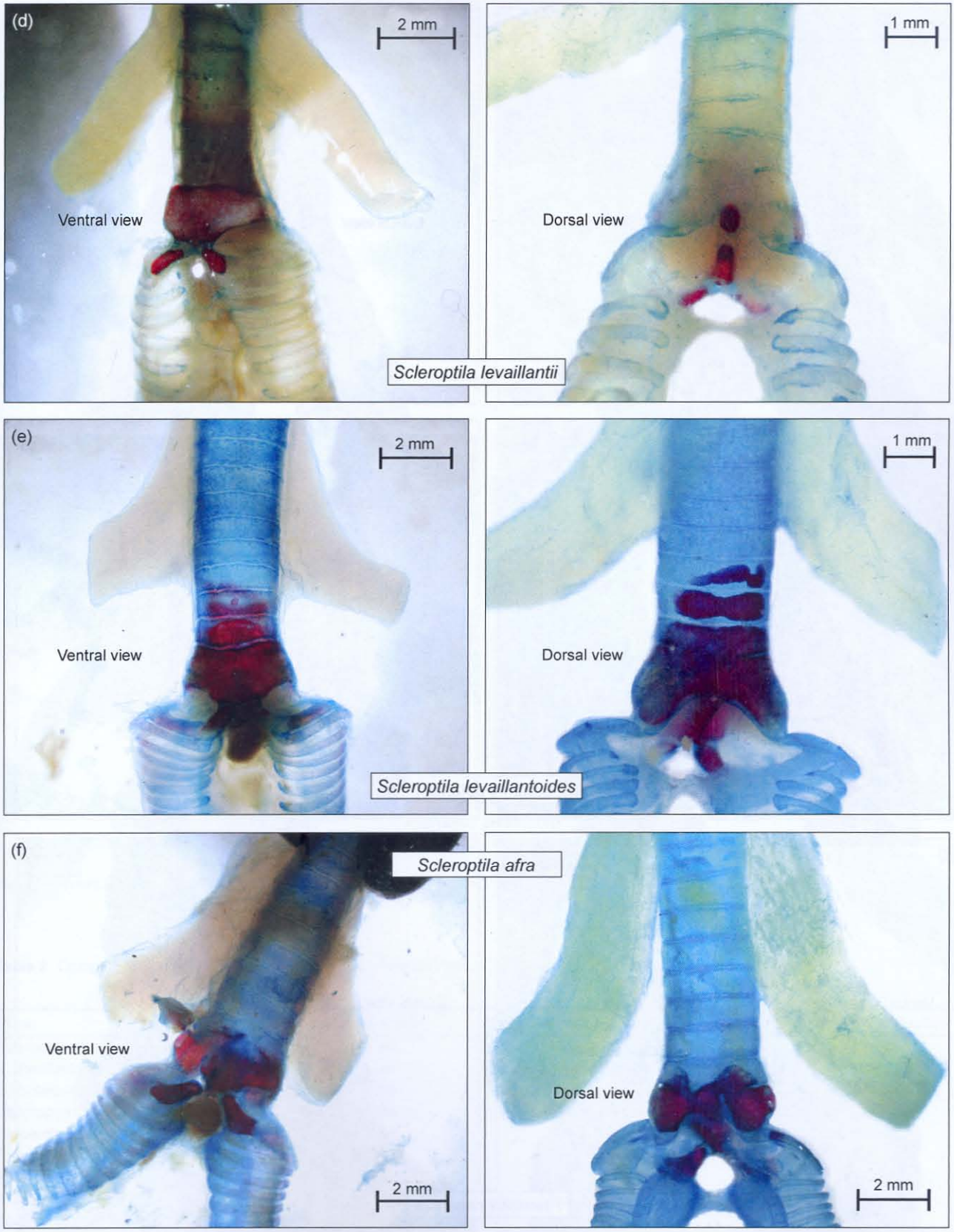

Figure 4: (cont.)

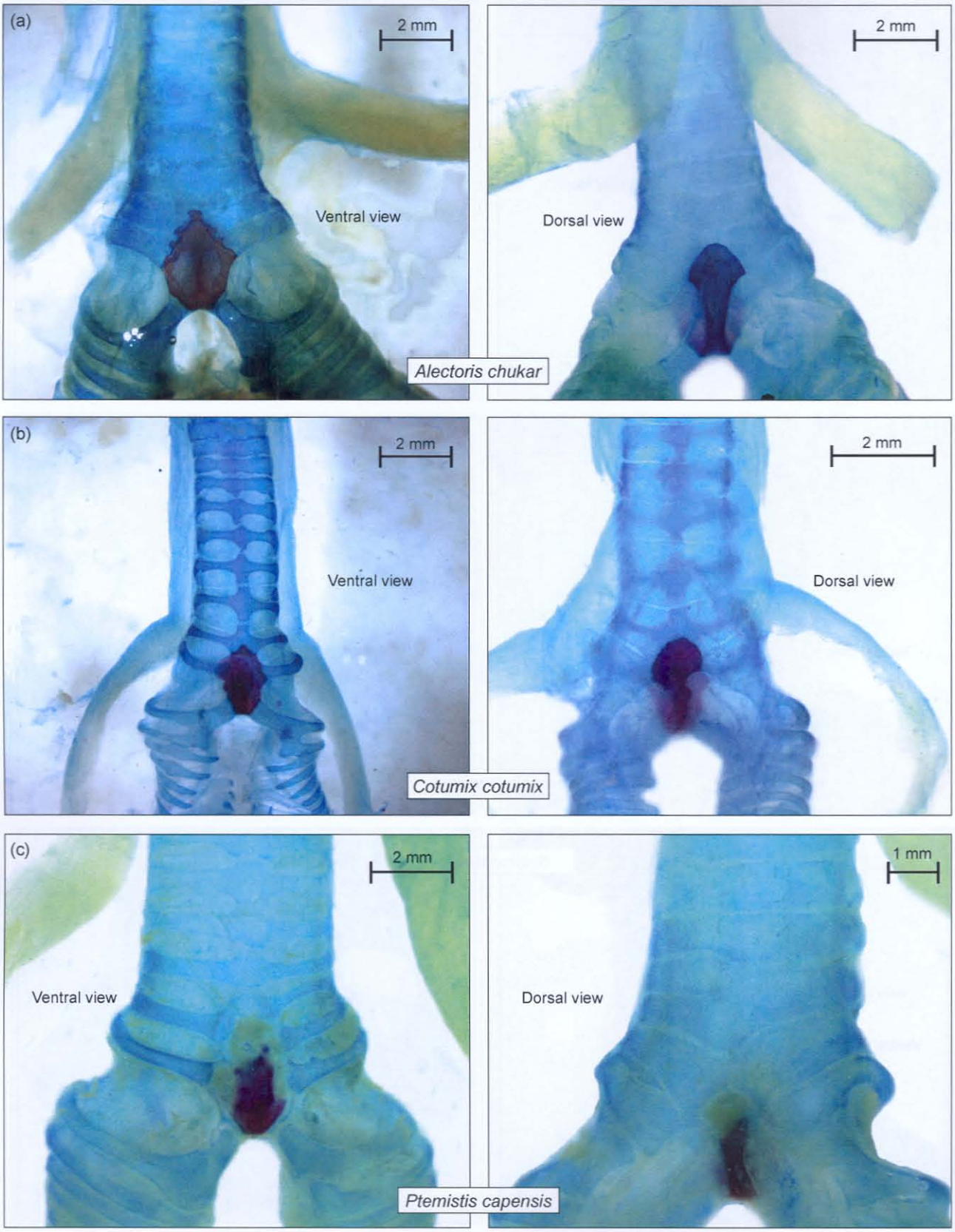

Figure 5: Ventral (left) and dorsal (right) views of the syringes of (a) *Alectoris chukar*, (b) *Coturnix coturnix*, (c) *Pternistis natalensis*, (d) *P. capensis* and (e) Swainson's Spurfowl *P. swainsonii*. Red colour = mineralised cartilage, blue = cartilage, transparent light brown = muscle

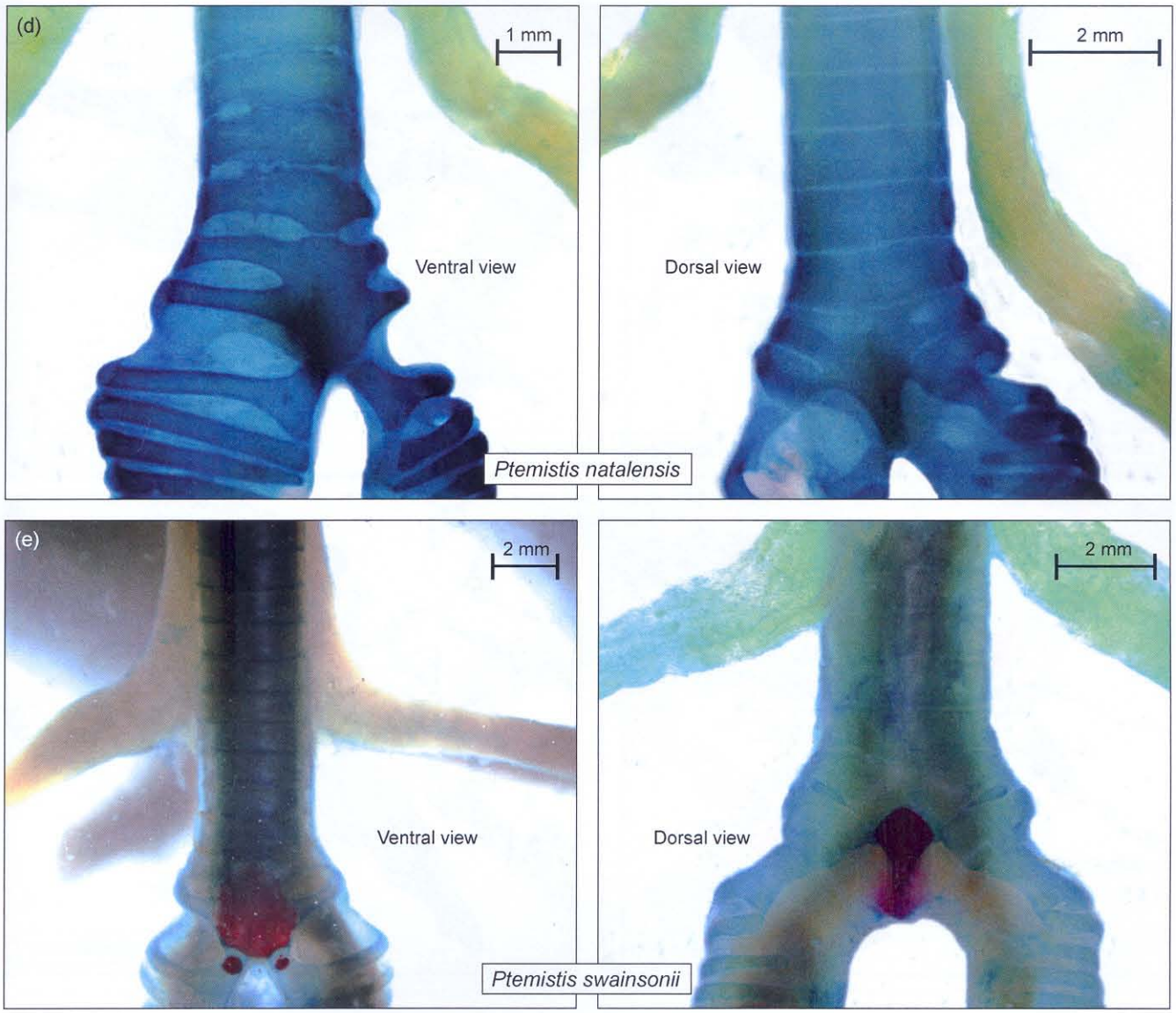

Figure 5: (cont.)

Table 2: Comparison of gross morphological features of the syringes

Taxon	Tympanum shape	Bronchial half ring 1 mineralisation	Tracheal ring mineralisation
Scleroptila afra	Shield-like	Mineralised	Non-mineralised
S. levaillantoides	Shield-like	Mineralised	Mineralised
S. levaillantii	Shield-like	Mineralised	Non-mineralised
Dendroperdix sephaena	Shield-like	Non-mineralised	Non-mineralised
Peliperdix coqui	Shield-like	Mineralised	Non-mineralised
P. capensis	Diamond	Non-mineralised	Non-mineralised
P. swainsonii	Diamond	Mineralised	Non-mineralised
P. natalensis	Diamond	Non-mineralised	Non-mineralised
Coturnix coturnix	Diamond	Non-mineralised	Non-mineralised
Alectoris chukar	Diamond	Non-mineralised	Non-mineralised
Numida meleagris	Shield-like	Mineralised	Mineralised
Gallus domesticus	Triangular	Mineralised	Mineralised

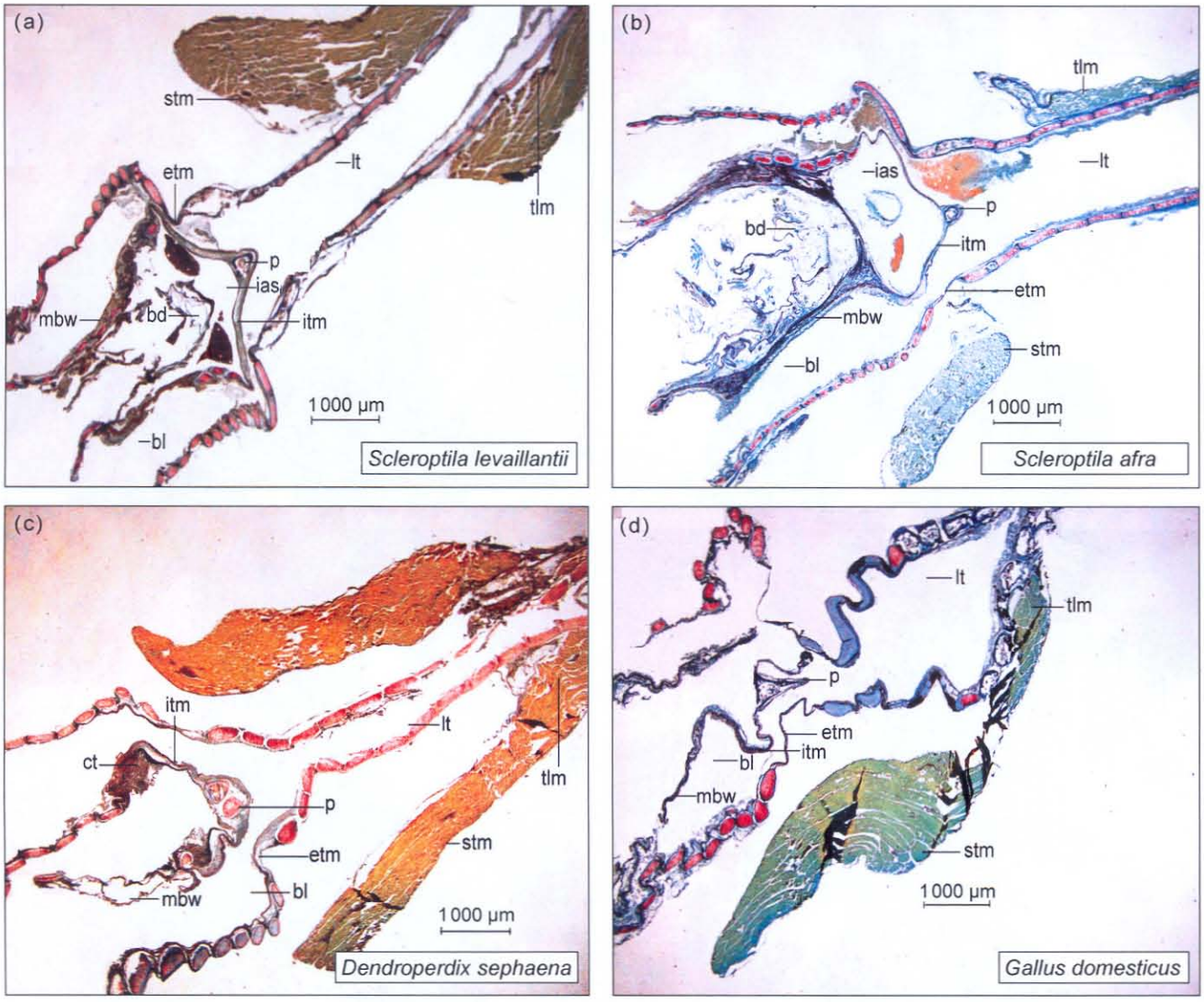

Figure 6: Histological structure of the syringes of francolins. (a) *Scleroptila levaillantii*, (b) *S. afra*, (c) *Dendroperdix sephaena*, (d) *Gallus domesticus*, (e) *Peliperdix capensis*, (f) *P. natalensis*, (g) *Coturnix coturnix* and (h) *Numida meleagris*. tlm = Tracheo-lateralis muscle, lt = lumen of trachea, ias = interclavicular air sac, ct = connective tissue, p = pessulus, itm = internal tympaniform membrane, etm = external tympaniform membrane, mbw = medial bronchial wall, bd = bronchiodesmus, bl = bronchial lumen, stm = sterno-trachealis muscle

could not at this stage translate to any coherent conclusion apart from the fact that a similar observation was made by other authors and Hogg (1982) thought this could have to do with conferring rigidity in the tracheal rings, which is an adaptation to vocalisation. With regard to the histology of the syringes, the features that separate francolins from spurfowls are based on the size of the pessulus. There is variation in shape of the pessulus, the presence/absence of the defined interclavicular air sac and the amount of connective tissue such that differences are observed even between the two spurfowl species. However, the amount of connective tissue puts species with tonal and whistling calls together (*S. levaillantii* and *S. afra*) from those that have atonal, raucous and grating calls (*P. capensis, P. natalensis* and *D. sephaena*). This feature could be related to differences in sounds between them, given that whistle-like sounds could be generated by shearing forces as a column of air is forced through a narrow aperture (see Gaunt et al. 1982), and the connective tissue could exert pressure to modify the lumen of the syrinx, which could be considered the column where air pass trough, generating the sound. At a first glance, this explanation would be contrary to what we would expect, given that spurfowls have thick connective tissue that would narrow their syrinx, but they have raucous calls. However, we know that the syrinx is only one component in a vocal system (Gaunt et al. 1982) and other factors may be modulating the final sound we hear.

This study had the goal to identify features that are consistent with the francolin–spurfowl dichotomy hypothesis. It would, however, be interesting to investigate the role of these features in voice production but unfortunately this was beyond the scope of this study.

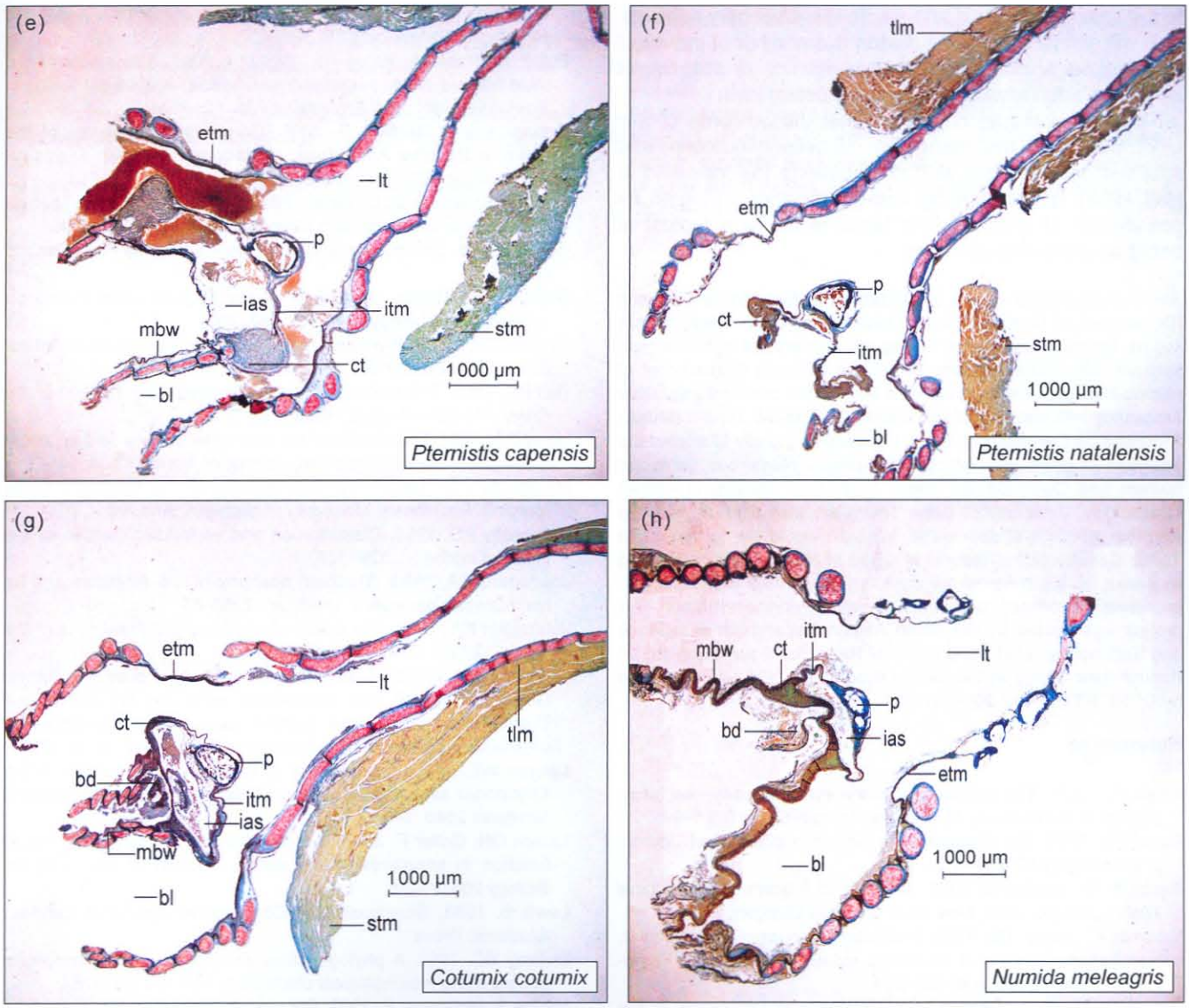

Figure 6: (cont.)

Table 3: Comparison of the histological features of syringes

Taxon	Pessulus shape and size	Interclavicular air sac[1]	Amount of connective tissue	External tympaniform membrane
Scleroptila afra	Rounded, small	Bound by internal tympaniform membrane	Thin	Shorter
S. levaillantii	Rounded, small	Bound by internal tympaniform membrane	Thin	Shorter
Dendroperdix sephaena	Rounded, larger	Absent	Thick	Shorter
Peliperdix capensis	Rounded, larger	Bound by internal tympaniform membrane	Thick	Shorter
P. natalensis	Triangular, larger	?	Moderate	Shorter
Coturnix coturnix	Triangular, larger	Bound by internal tympaniform membrane	Thin	Shorter
Numida meleagris	Rounded, small	Bound by internal tympaniform membrane	Thin	Shorter
Gallus domesticus	Triangular, larger	Absent	Thin	Long

[1] ? = Presence or absence of feature could not be determined from distorted tissue section

Conclusions

A number of gross morphological and histological features were identified as differentiating francolins from spurfowls (though with exceptions mainly in *D. sephaena*). What emerged as an area for future research was investigation into the role of each part in shaping francolin and spurfowl vocalisations also including other galliform species in addition

to the sampled francolin and spurfowl evolutionary relatives. This will definitely require a reasonable number of individual samples per species so that any presence or absence of intra-/inter-specific variations could be determined.

Finally, it could be concluded that the outcome of this work points to the distinction between francolin and spurfowl assemblages with *D. sephaena* (as indicated in Hall 1963) still presenting some difficulties owing to its possession of characteristic features that are typical of both francolins and spurfowls.

Acknowledgements — We are indebted to Mrs Morea Peterson (Department of Human Biology, University of Cape Town) for her superb technical assistance during the preparation of histological sections. Ms Barbara Moore and Barbara Young (Department of Human Biology, University of Cape Town) also provided invaluable assistance with histological procedures. Dr Pablo L Tubaro (Museo Argentino de Ciencias Naturales 'B Rivadavia') kindly facilitated the analyses of gross morphology of syringes. Numerous gamebird hunters and Owen Davies (Percy FitzPatrick Institute of African Ornithology, University of Cape Town) are also thanked for help with the collection of specimens. We also would like to thank Mrs Tamar Cassidy of the National Museum of Natural History (Ditsong Museums of South Africa) for providing us with the whole alcohol-preserved specimens from which we extracted the syringes. This project was funded by the South African Department of Science and Technology (DST) and National Research Foundation (NRF) through their Centre of Excellence Programme. CK was supported by CONICET PIP 112-200801-0074.

References

Ames PJ. 1971. The morphology of the syrinx in passerine birds. *Bulletin of the Peabody Museum of Natural History* 37: 1–94.

Appel FW. 1929. Sex dimorphism in the syrinx of the Fowl. *Journal of Morphology* 47: 497–517.

Bancroft JD, Gamble M. 2002. *Theory and Practice of Histological Techniques* (5th edn). New York: Churchill Livingstone.

Bloomer P, Crowe TM. 1998. Francolin phylogenetics: molecular, morphobehavioral, and combined evidence. *Molecular Phylogenetics and Evolution* 9: 236–254.

Brown C, Ward D. 1990. The morphology of the syrinx in the Charadriiformes (Aves): possible phylogenetic implications. *Bonner Zoologische Beiträge* 41: 95–107.

Cannell PF. 1988. Techniques for the study of avian syrinxes. *Wilson Bulletin* 10: 289–293.

Crowe TM, Bowie RCK, Bloomer P, Mandiwana TG, Hedderson TAJ, Randi E, Pereira SL, Wakeling J. 2006. Phylogenetics, biogeography and classification of, and character evolution in, gamebirds (Aves: Galliformes): effects of character exclusion, data partitioning and missing data. *Cladistics* 22: 1–38.

Crowe TM, Crowe AA. 1985. The genus *Francolinus* as a model for avian evolution and biogeography in Africa. In: Schuchmann KL (ed.), *Proceedings of the International Symposium on African Vertebrates*. Bonn: Museum Alexander Koenig. pp 207–231.

Crowe TM, Harley EH, Jakutowicz MB, Komen J, Crowe AA. 1992. Phylogenetic, taxonomic and biogeographical implications of genetic, morphological, and behavioural variation in francolins (Phasianidae. *Francolinus*). *Auk* 109: 24–42.

Delacour J, Mayr E. 1945. The family Anatidae. *Wilson Bulletin* 57: 3–55.

Farais IP, Ortí G, Meyer A. 2000. Total evidence: molecules, and the phylogenetics of cichlid fishes. *Journal of Experimental Zoology* (*Molecular and Developmental Evolution*) 288: 76–92.

Frank T, Walter I, Probst A, König HE. 2006. Histological aspects of the syrinx of the male Mallard (*Anas platyrhynchos*). *Anatomia, Histologia, Embryologia* 35: 396–401.

Frank T, Probst A, König HE, Walter I. 2007. The syrinx of the male Mallard (*Anas platyrhynchos*): special anatomical features. *Anatomia, Histologia, Embryologia* 36: 121–126.

Gaban-Lima R, Höfling E. 2006. Comparative anatomy of the syrinx in the tribe Arini (Aves: Psittacidae). *Brazilian Journal of Morphological Sciences* 23: 501–512.

Gaunt AS, Gaunt SLL, Casey RM. 1982. Syringeal mechanics reassessed> evidence from *Streptopelia*. *Auk* 99: 474–494.

Gill FB. 1990. *Ornithology* (2nd edn). New York: WH Freeman and Company.

Griffiths CS. 1994a. Monophyly of the Falconiformes based on syringeal morphology. *Auk* 111: 787–805.

Griffiths CS. 1994b. Syringeal morphology and the phylogeny of the Falconidae. *Condor* 96: 127–140.

Hall BP. 1963. The francolins, a study in speciation. *Bulletin of the British Museum* (*Natural History*) 10: 8–204.

Hogg DA. 1982. Ossification of the laryngeal, trachea and syringeal cartilages in the domestic fowl. *Journal of Anatomy* 134: 57–71.

Humphrey PS. 1955. The relationships of the seaducks (tribe Mergini). PhD thesis, University of Michigan, Ann Arbor, USA.

Humphrey PS. 1958. Classification and systematic position of the elders. *Condor* 60: 129–155.

Johnsgard PA. 1961. Tracheal anatomy of the Anatidae and its taxonomics significance. *Wildfowl* 12: 58–69.

Johnsgard PA. 1988. *The Quails, Partridges, and Francolins of the World*. Oxford: Oxford University Press.

Krakauer AH, Tyrrell M, Lehmann K, Losin N, Goller F, Patricelli GL. 2009. Vocal and anatomical evidence for two-voiced sound production in the greater sage-grouse *Centrocercus urophasianus*. *Journal of Experimental Biology* 212: 3719–3727.

Lanyon WE. 1986. A phylogeny of the thirty-three genera in the *Empidonax* assemblage of tyrant flycatchers. *American Museum Novitates* 2846: 1–64.

Larsen ON, Goller F. 2002. Direct observation of syringeal muscle function in songbirds and a parrot. *Journal of Experimental Biology* 205: 25–35.

Lewis B. 1983. *Bioacoustics: a Comparative Approach*. London: Academic Press.

Livezey BC. 1986. A phylogenetical analysis of recent Anseriform genera using morphological characters. *Auk* 103: 737–754.

Madge S, McGowan P. 2002. *Pheasants, Partridges, and Grouse: a Guide to the Pheasants, Partridges, Quails, Grouse, Guineafowl, Buttonquails, and Sandgrouse of the World*. Princeton: Princeton University Press.

McGowan PJK. 1994. Family Phasianidae (pheasants and partridges). In: del Hoyo J, Elliott A, Sargatal J (eds), *Handbook of the Birds of the World, Vol. 2: New World Vultures to Guineafowls*. Barcelona: Lynx Edicions.

Milstein P le S, Wolff SW. 1987. The over-simplification of our "francolins". *South African Journal of Wildlife Research* Suppl. 1: 58–65.

Myers JA. 1917. Studies on the syrinx of *Gallus domesticus*. *Journal of Morphology* 20: 165–215.

Mobley JA, Prum RO. 1995. Phylogenetic relationships of the Cinnamon tyrant, *Neopipo cinnamomea*, to the Tyrant flycatchers (Tyrannidae). *Condor* 97: 650–662.

Pruett CL, Winker K. 2010. Alaska Song Sparrows (*Melospiza melodic*) demonstrate that genetic marker and method of analysis matter in subspecies assessments. *American Ornithologists' Union* 67: 162–171.

Prum RO. 1992. Syringeal morphology, phylogeny, and evolution of the Neotropical manakins (Aves: Pipridae). *American Museum Novitates* 3043.

Prum RO, Lanyon WE. 1989. Monophyly and phylogeny of the

Schiffornis group (Tyrannoidea). *Condor* 91: 444–461.

Seller TJ. 1983. Control of sound production in birds. In: Lewis B (ed.), *Bioacoustics: a Comparative Approach*. London: Academic Press. pp 93–124.

Steven P, Paulsen F, Tillmann B. 2000. Orcein-picroindigocarmine – a new multiple stain. *Archives of Histology and Cytology* 63: 397–400.

Templeton AR. 1989. The meaning of species and speciation: a genetic perspective. In: Otte D, Endler JA (eds), *Speciation and its Consequences*. Sunderland, Massachusetts: Sinauer Associates. pp 3–27.

Tsukahara N, Yang Q, Sugita S. 2008. Structure of the syringeal muscles in jungle crow (*Corvus macrorhynchus*). *Anatomical Science International* 83: 152–158.

Zimmer KJ, Robbins MB, Kopuchian C. 2008. Taxonomy, vocalizations, syringeal morphology and natural history of *Automolus roraimae* (Furnariidae). *Bulletin of the British Ornithologists' Club* 128: 14–33.

Received November 2010, accepted April 2011
Editor: A Monadjem