The phylogenetic significance of the morphology of the syrinx, hyoid and larynx, of the Southern Cassowary, Casuarius casuarius (Aves, Palaeognathae)

Phoebe Louise McInerney (phoebe.mcinerney@flinders.edu.au)
Flinders University https://orcid.org/0000-0001-7224-8636
Mike S. Y. Lee
Flinders University
Alice M. Clement
Flinders University
Trevor H. Worthy
Flinders University

Research article

Keywords: Palaeognathae, Cassowary, Syrinx, Hyoid, Larynx, Morphology, Phylogenetics, Optimisation

Posted Date: August 5th, 2019

DOI: https://doi.org/10.21203/rs.2.12067/v1

Version of Record: A version of this preprint was published on December 27th, 2019. See the published version at https://doi.org/10.1186/s12862-019-1544-7.

Abstract

The Palaeognathae are a basal clade within Aves and include the large and flightless ratites and the smaller, volant tinamous. Although much research has been conducted on various aspects of palaeognath morphology, ecology, and evolutionary history, there are still areas which require investigation. This study aimed to fill gaps in our knowledge of the Southern cassowary, Casuarius casuarius, for which information on the syrinx, hyoid and larynx is lacking despite these structures having been recognised as performing key functional roles associated with vocalisation, respiration and feeding. Previous research into the syrinx and hyoid have also indicated these structures to be valuable for determining evolutionary relationships among neognath taxa, and thus may also shed light on palaeognath phylogeny, which still exhibits strong conflict between morphological and molecular trees. We thus documented variation across palaeognaths in syringeal, hyoidal, and laryngeal character states, using both the literature and novel new observations (e.g. of cassowary). Notably the molecular moa-tinamou clade was found to share derived morphological traits including the ossification of the cricoid and arytenoid cartilages, and an additional cranial character, the articulation between the maxillary process of the nasal and the maxilla. Syringeal, hyoidal and laryngeal characters better optimised onto the topology resulting from phylogenetic analyses of a combined molecular and morphology analysis, than molecular-only or morphological-only trees. One primary factor for this support was the aforementioned shared character states between the moa and tinamou, also present in Lithornis and outgroup taxa.

Background

Palaeognathae is one of two primary avian clades. Considered to have diverged during the middle Cretaceous [1, 2, 3], Palaeognathae comprises the volant tinamous (Tinamidae) (South America) and the flightless, cursorial ratites such as the Australian emu (*Dromaius*) and the extinct New Zealand Moa (Dinornithiformes) [2, 4 p. 272, 5 - 7]. The large, cursorial *Casuarius casuarius,* the southern cassowary, forms Casuariidae with the Australian emu and is nested within Palaeognathae [8]. The southern cassowary is endemic to the tropical rainforests of New Guinea and Australia [8, 9]. It has a solitary nature [10] and a preference for dense forested habitats [11], hence relatively little is known about cassowary ecology in comparison to its extant relatives [12]. These gaps in knowledge extend to the phenotype: poorly studied structures in the cassowary include the syrinx, hyoid and larynx, despite morphological and comparative analyses of these structures in other palaeognaths, and their importance for primary biological functions and potentially phylogenetic inferences.

Birds primarily vocalise through the movement and manipulation of syringeal elements within the syrinx, and so vocal output is constrained by the mechanical design of this organ within the vocal tract [13, 14, 15, 16]. The syrinx has been described for numerous taxa, revealing substantial morphological variation, often reflecting differential vocalisation demands [17]. Due to the simplicity of palaeognath syrinx structures, nineteenth century zoologists claimed ratites lacked syringeal characteristics, and therefore, had no syringeal organ. However, in the late 1800s this assumption was challenged, with Forbes [18]

proving the presence of several syringeal structures, such the tympaniform membranes. Undoubtedly though, palaeognaths have structurally very simple syrinxes in comparison to those of many neognaths [19 p. 123], leading to suggestions that they represent either an example of evolutionary degeneration and/or retention of an unspecialised and primitive form [18, 19 p. 123, 20 p. 60, 65]. The apparent simplicity of the organ may have contributed to the cassowary syrinx receiving only superficial study [18, 19, 20]; an absence of detailed comparative studies with other palaeognath taxa is also notable.

In Aves, the laryngeal and hyoid apparatus form the floor of the oropharyngeal cavity [22, 23 p. 50], sitting directly beneath the mandible [24 p. 386]. As individual structures comprising their own skeleton and complex musculature, both work independently and in synchrony to facilitate respiration and consumption [22, 23 p. 50, 25, 26 p. 69]. The hyoid apparatus forms an essential structural element within the upper digestive tract, supporting and controlling the lingual corpus, the tongue [22, 23 p. 51, 26 p. 77, 27, 28]. The larynx attaches to the dorsal aspect of the hyoid corpus, and acts as the gateway into the trachea, a barrier to foreign bodies entering the respiratory tract during swallowing [23 p. 50, 25, 26 p. 77].

The literature on the syrinx, hyoid apparatus and larynx demonstrates that these organs not only play key functional roles but exhibit considerable variation in morphology between taxa [25, 29]. Thus, descriptive analyses for individual taxa is essential to the development of a comprehensive understanding of the organs [25]. The syrinx and hyoid have also proven valuable in phylogenetic inference among various neognath clades, including the suboscine family Rhinocryptidae [30], providing novel phylogenetic characters, informing relationships and assisting in the classification of taxa by morphologists and systematists [6, 30 - 35]. Therefore, it is likely analysis of these structures could be similarly beneficial within palaeognath phylogenetics.

The hyoid apparatus and syrinx of the cassowary have previously been described, primarily during the late 1800s and 1900s [for example 18, 19, 21, 36]. However, these descriptions are brief and often lack context, partly due to authors not having the technology now available. Furthermore, there is no description of the cassowary larynx, despite it being described for other palaeognaths. Clearly, further morphological analysis of these structures is desirable and may prove phylogenetically important [6, 31 – 34, 37], as found for aforementioned neognath clade, Rhinocryptidae [30]. Phylogenetic analyses of palaeognaths have revealed discordant topologies between morphological and molecular data, thought to be a result of convergent morphological traits, with genomic data largely driving the current consensus [1, 3, 5, 7, 38]. Research has identified few morphological characters in support of relationships found in molecular-based analyses; most are cranial characters, not associated with structures related to cursorality and flight which might be susceptible to convergence [2, 38]. This suggests analysis of overlooked structures unrelated to locomotion, such as the syrinx, hyoid apparatus and larynx, may also retrieve evolutionary patterns more similar to that of the molecular evolutionary tree for palaeognath taxa. Thus, we herein present new anatomical observations and comparative analyses of the syrinx, hyoid, and larynx (SHL) of the Southern Cassowary, *Casuarius casuarius,* and identify morphological characters within these structural systems which contribute to improved resolution of phylogenetic relationships.

Results

Syrinx

Figure 1

Palaeognath syringeal elements with tracheosyringeal cartilages differentiated by brown shading (B-E), interannular tissues are shaded in grey. A-B, Cassowary, *Casuarius casuarius*, FUR180; ventral, scale bar = 10mm. **C**, Tinamou, *Nothura darwinii*, adapted from Garitano-Zavala [39, fig. 1C]; ventral. **D**, Rhea, *Rhea americana,* adapted from Forbes [18, fig. 7, 8]; ventral and dorsal. **E**, Kiwi, *Apteryx mantelli,* adapted from Forbes [18, fig. 3], ventral. **F**, Ostrich, *Struthio camelus*, adapted from Forbes [18, fig. 1]; ventral. Abbreviations: bs.cs: bronchosyringeal cartilages, ia.i: interannular interval, tr.c: tracheal cartilages, trs.cs: tracheosyringeal cartilages. C-F scaled to same size approximately.

The cartilaginous syrinx of our female cassowary (FUR180) structurally conforms to the tracheo-bronchial syrinx type (figure 1A-B); as do all palaeognaths [20 p. 61]. The syrinx of FUR180 is simple and comparable in form with others previously described, such as the adult male assessed by King [19 p. 124], indicating the absence of sexual dimorphism in this organ. Of the palaeognaths, and all birds, the ostrich has been considered to have one of the simplest syrinxes, with which it can produce a limited repertoire of sounds (figure 1F) [40]. However, the ostrich syrinx does have a pessuliform process and potentially a tympanum [18, 41], indicating a more derived state than in other palaeognaths including the cassowary. The syrinx of the rhea (figure 1D) is the most complex, due to the presence of specialised anatomical structures, including fully developed intrinsic musculature, absent in most other palaeognaths [18, 21, 42].

Cart. tracheosyringeales- There are five tracheosyringeal cartilages associated with the cassowary syrinx, distinct from preceding true tracheal cartilages as they are thinner ventrally and laterally. Dorsally all are incomplete along the midline, with the extremities bending medially towards the centre of the syrinx, a result of tracheosyringeal membranes contracting post-death [21]. Forbes [18] and Pycraft [21] noted the presence of these imperfect tracheosyringeal cartilages, proposing the cranio-caudal space formed between the cartilage extremities is occupied by transversely running fibrous and elastic tissues [21] later termed the tracheosyringeal membranes (*mem. tracheosyringealis*) [19 p. 128]. Dorsally incomplete cartilages are present in the ostrich, moa, kiwi and emu, although lacking in the rhea and tinamou. The number of incomplete cartilages varies depending on the species, although no other species approaches having nine, the number present in the cassowary specimen. Three have been noted for the moa [43] and emu [18, 21], and two for the ostrich [18]. The number present in the kiwi is dependent on the species; *Apteryx australis* has three compared to the single incomplete cartilage in *A. mantelli* [18].

All five tracheosyringeal cartilages angle caudally along the medial line of the ventral side of the cassowary syrinx, with the degree of the angle increasing caudally with each cartilage, paired with a cranio-caudal increase of width. These features are common in palaeognaths, although variable in some taxa such as the ostrich and kiwi, which develop this character on the dorsal side of the syrinx. Ventral modification to the most caudal tracheosyringeal cartilages in the moa differentiate this taxon from others: the cartilage lengthens cranio-caudally at the most caudal point of the V, from which a caudo-medially directed projection extends [43]. Oliver [43] interpreted this keel syringeal ring to likely be the ventral attachment point of a pessulus.

Noted as a common feature among casuariids (*Dromaius* and *Casuarius*) by Pycraft [21], the cassowary syrinx shows poor transitional definition between bronchosyringeal and tracheosyringeal cartilages. The most caudal tracheosyringeal cartilage (trs. cs. 1) closely reflects the structure of the first bronchosyringeal cartilage with cartilages differentiated only by partial fusion of the ventral extremities present in the former, maintaining a single element structure. Tinamous also display a gradual transition between cartilage types [39], although greater transitional definition is noted in the ostrich, kiwi and rhea. The ostrich trachea increases in diameter in the few cartilages preceding tracheal bifurcation; the following bronchosyringeal cartilages are much narrower craniocaudally [18, 41]. Alternatively, distinction between the two cartilage types in kiwi is formed from a widening of the bronchosyringeal cartilages after tracheal bifurcation [18, 21]. The transitional definition in the rhea is unique among palaeognaths with the fusion of tracheosyringeal cartilages forming a tympanum just cranial to tracheal bifurcation [18, 21, 42].

Pessulus- No pessulus is present in the cassowary specimen FUR180, with the left and right medial tympaniform membranes fusing transversely along the dorso-ventral plane, at the level of tracheal bifurcation. As expected, no intrinsic musculature was found in the µCT-generated model as expected given its absence in specimens described by Forbes [18] and Pycraft [21]. Similarly, the tinamous [39], kiwi [18, 21] and emu [18] also lack a pessulus. In the rhea, this structure is present; the pessulus links the caudo-medial point of the dorsal and ventral sides of the tympanum as a narrow osseous bridge [18]. Ossification has been recorded for rhea, despite the structure being primarily cartilaginous, suggesting that increased ossification occurs later in ontogenetic staged in males. Although we found no support for this in the NMNZ collection, based on a described structure by Owen [44], Oliver [43] reported that moa also develop an ossified pessulus forming a partial bridge across the ring and likely completed by cartilage. Early observations of the ostrich syrinx found the third tracheosyringeal cartilage to contain a short caudal projection, medially on the ventral border [18]. This is considered a pessuliform process; not a true pessulus due to it not traversing the ventrodorsal width of the trachea. Yildiz and colleagues [41] noted the presence of a double-folded structure formed of connective tissue, suggesting this may act similarly to a true pessulus, providing support for the medial tympaniform membranes.

Tympanum- FUR180 has complete lack of fusion between tracheosyringeal cartilages indicating the tympanum is absent in cassowaries. No evidence was found to support Forbes' [18] claim for the

presence of an ‘expanded’ tympanum, and it is unlikely that incomplete and unfused cartilages such as we observe could function similarly to a true tympanum.

Of all palaeognath taxa, a tympanum has been described in the ostrich, rhea, and moa; this structure is absent in tinamous [39]. However, among these taxa, only in rhea has the presence been confirmed with complete dorsal and ventral fusion of four to six tracheosyringeal cartilages [18, 21, 42]. Fusion between tracheosyringeal elements has been described in some ostrich specimens, with the tympanum comprising three tracheal cartilages, although Yildiz *et al.* [41] found the cartilages only appeared to be fused through the presence of ligamentum annulare. Oliver [43] also provides a description pertaining to the presence of a possible tympanum in moas, located cranial to the tracheosyringeal cartilages [45 p. 107]. We searched numerous moa specimens and provide in the supplementary data (SI.1) a list by species and presence of tracheal rings, noting the type of rings present including the unique syringeal keeled ring that represents an incomplete ossified pessulus. The tympanum as described by Oliver [43, fig 26] was not found among any tracheal ring sets present in any moa taxa (SI. 1.). We consider that given the keeled syringeal ring is invariably present, then there was no tympanum in moa, given such would incorporate this ring with more than one other, making an even more robust ossified element.

Cart. bronchosyringeales- The cassowary bronchi are asymmetrical, with the left bronchus larger in diameter. Also contributing the asymmetry, the ventral extremity of the third bronchosyringeal cartilage on the left bronchi is angled ventro-medially and expands caudally, both characters are absent on the right bronchi, although both sides display an increase in cartilage length from preceding cartilages. The medial extremities of the bronchosyringeal cartilages overlap dorsally and ventrally, indicating a potential for expansion of interannular intervals during use. Interannular intervals (spaces) between bronchosyringeal cartilages remain relatively uniform for the length of the specimen, with the absence of large, paired intervals indicating lateral tympaniform membranes do not develop. The kiwi similarly possesses uniform intervals [18], whereas in the ostrich, ventral intervals narrow, and cranial intervals widen [18, 41]. The tinamou possesses two wide interannular intervals between the first to the fourth bronchosyringeal cartilages [39]. The first two bronchosyringeal intervals indicate the presence of the lateral tympaniform membrane, also noted for the rhea.

Hyoid

Figure 2

Palaeognath hyoid elements. A-B, Cassowary, *Casuarius casuarius*, FUR180; dorsal view, A- scale bar = 10mm. **C**, Tinamou, *Nothoprocta perdicaria*, NMNZ S. 22983; dorsal view, scale bar = 10mm. **D**, Moa, *Megalapteryx*, NMNZ S. 400; dorso-lateral view, scale bar = 10mm. **E**, Emu, *Dromaius novaehollandiae,* adapted from Parker [36, plate XII]; dorsal view. **F**, Rhea, *Rhea americana,* adapted from Parker [36, plate

X]; dorsal view. **G**, Ostrich, *Struthio camelus*, adapted from Soley *et al.* [22, fig. 3]; dorsal view. Abbreviations: bh: basihyal, buh, basiurohyal, cb: ceratobranchial, epb, epibranchial, ur: urohyal.

All typical hyoid skeletal components are present within the hyoid apparatus of FUR180 (figure 2A-B). The basihyal, urohyal, and a paraglossal are cartilaginous, with only the ceratobranchials ossified. Ossification of ceratobranchials is common to all Aves, while the extent to which other elements ossify varies by lineage [22, 24 p. 367, 45 p. 110, 46, 47].

Basiurohyal- The joint between the basihyal and urohyal is indiscernible, with complete fusion of the two cartilaginous skeletal elements forming the basiurohyal (figure 2B). This character is common among palaeognaths; the only exception is the rhea where the urohyal is lost (figure 2F) [24 p. 369, 36, 48]. Rostrally, the basiurohyal curves dorsally, preceding a slight ventral arch centrally along the corpus, with the caudal point terminating with a minor inwards hook towards the laryngeal cricoid cartilage. The basiurohyal in the cassowary and emu have rounded tips [27], compared to that of the ostrich (figure 2G) which tapers caudally to a pointed tip, and rostrally terminates in two bulbous projections divided by a shallow notch [22, 27, 36]. The basihyal of the rhea is more cylindrical than in other palaeognaths although it also terminates in a rounded rostral tip [24 p. 366, 48].

Despite the basiurohyal being completely unossified in the cassowary, ossification of basiurohyal elements does occur in the kiwi [21] and rhea [48]. A partially ossified basiurohyal was also identified in a single moa specimen (*Megalapteryx*, specimen no. NMNZ S.400). The identification of an ossified moa basiurohyal shows that partial ossification may exist in at least the basal moa genus *Megalapteryx* though a lack of these elements in the moa fossil record limit our ability to determine whether this is an incident cause by the age of the specimen. We also identified a basiurohyal in which the urohyal and caudal portion of the basihyal were ossified in a tinamou (figure 2C) (*Nothoprocta perthicaria*, NMNZ S.22983). In an earlier study, Li *et al.* [47] found that despite midline ossification being a key component of the hyoid apparatus in neognath birds, the degree of ossification in palaeognaths varies and is often incomplete when present. This is correct for most palaeognaths, however even our limited observation of tinamou and moa specimens show complete ossification can be present. Therefore, further investigations across a wider sample of taxa is required to fully assess this variability.

Ceratobranchiale and epibranchiale- The basiurohyal articulates mid-way along its lateral edges with the ceratobranchials. The bulbous, disk-like proximal ends of the ceratobranchials sit low in the concave basiurohyal sockets; the articular surface of the sockets is larger than that of the proximal ceratobranchial end, indicating an allowance for considerable movement within the joint. The ceratobranchials are elongate with one third of their length extending past the caudal point of the basiurohyal. The shaft curves dorsally towards the ceratobranchial-epibranchial joint, from which the shorter cartilaginous epibranchials extend caudo-dorsally. In our specimen the right epibranchial is deformed with a large, sharp medial bend and increased tissue mass, contrasting with the smooth curve of the left epibranchial. The lack of symmetry, as well as no previous mention of such deformity within

the literature for the cassowary or any other palaeognath taxa, indicates a pathology. The shape would impede the functionality of the epibranchial, including movement in and out of the hyoid sheath.

Palaeognath ceratobranchials are often cylindrical in shape, although can be slightly flattened as in the rhea [36]. Ostrich epibranchials are elongate [24 p. 371] compared to most other palaeognaths, which have short epibranchials relative to the ceratobranchials [24 p. 367]. In the tinamou, both epibranchials and ceratobranchials display increased elongation [36] compared to other taxa. We found the tinamou to have ossified epibranchials, differing from other palaeognaths including its closest relative, the moa, in which the epibranchials do not ossify (figure 2D).

Paraglossum- The cranial portion of the hyoid skeleton attaches to the paraglossal; both structures are encased by soft tissue, connecting the paraglossal to the back of the tongue body. The cassowary paraglossal is a single un-ossified element seemingly with a rounded triangular shape, as suggested by Parker [36]. The paraglossal of the emu (figure 2E) is similarly tear-drop shaped, although the caudal edge may be rounded or scalloped [27, 36, 49]. The shape of the paraglossal in the rhea reflects the more triangular shape of the tongue, although it is smaller and with an oval opening dorsal on the palate [48]. The tinamou paraglossal is much narrower in width than other species, with scalloped margins and two caudally directed projections, one from each caudo-lateral corner [24 p. 372, 36]. Unique among palaeognaths, the ostrich paraglossal is divided into two narrow, caudo-laterally directed individual paraglossia, situated ventro-laterally in the tongue body [24 p. 371, 27, 50]. As the ostrich is phylogenetically basal among palaeognaths, this paired state could potentially be plesiomorphic, the rhea then shows partial fusion and the other palaeognaths, complete fusion.

Lingual corpus- When compared to the tongues of other avian taxa, palaeognath tongues are significantly shorter relative to the mandible; they have thus been described as vestigial organs, rudimentary in morphology [28, 50]. The cassowary tongue is no exception, reflecting the limited role played by the tongue during the ‘catch and throw’ feeding method, a method utilising obligate inertial feeding in which the tongue is unrequired [23 pp. 53 and 74, 51, 52].

As in the rhea, emu, and ostrich (24 pp. 366, 370 and 371, 28, 48, 49, 53], the cassowary tongue is cranio-caudally flattened and triangular. Only the tongue of the kiwi varies significantly, being elongate [54], reflecting the shape of the long and narrow bill. The kiwi elongate tongue is likely a result of dietary specialisation, with the kiwi diet consisting primarily of invertebrates [55]; the elongated bill is required for detecting buried or submerged prey using vibration-sensitive mechanoreceptors [56]. The cassowary tongue corpus has a smooth and rounded rostral apex as in tinamous and ostriches, although varying from the pointed tip of the rhea tongue [24 p. 372, 48]. The caudal and rostral edges of the tongue are concave, although the rostral notch is more prevalent in the rhea, ostrich, and tinamou, than the cassowary and emu [24 p. 372, 48, 57].

Numerous lingual papillae, arranged asymmetrically, line the sides of the cassowary tongue, increasing in length and width caudally towards the tongue base. Although the emu displays analogous structures, these are not present in all palaeognaths. The tongue of the rhea lacks lateral papillae although the

caudo-lateral corners project caudally [28, 49, 48, 57]. Both absence and presence of papillae have been noted for the ostrich [24 p. 371, 27], and papillae are completely absent in tinamous [24 p. 372]. Only the emu tongue is considered to have caudal papillae, although poorly defined and rudimentary when compared to those directed laterally [29, 49]. Lingual papillae are absent on the dorsal surfaces of all palaeognathous tongues [28].

Larynx

Figure 3

Palaeognath laryngeal elements. A-B, Cassowary, *Casuarius casuarius*, FUR180; dorsal view, scale bar = 10mm. **C**, Rhea, *Rhea americana,* adapted from Crole and Soley [48, fig. 7]; dorsal view. **D**, Ostrich, *Struthio camelus*, adapted from Tadjalli [46, fig. 9a, b]; dorsal view. **E**, Tinamou, *Nothura maculosa*, MMC321; ventral view. **F**, Moa, *Euryapteryx curtus*, NMNZ S. 44757; lateral and ventral views. **G**, Kiwi, *Apteryx rowi*, NMNZ OR.27243A; dorsal view. Abbreviations: ar: arytenoids, cr.c: cricoid cartilage, cr.w: cricoid wings, oss: ossification, pc: procricoid, tr.c: tracheal cartilages.

The larynx of the cassowary FUR180 (figure 3A-B) has a standard avian anatomy, composed of the cartilaginous skeletal elements, the cricoid, procricoid, and paired arytenoid cartilages. FUR180 is entirely cartilaginous as in the emu, some palaeognaths (ostrich [46], rhea [48] and kiwi) have poorly and variably defined ossification centres in the corpus, with partial ossification in the rhea [48] and kiwi (personal observation, figure 3) likely dependent on ontogenetic stage. Only tinamous and moa have a strongly ossified cricoid corpus where the entire corpus is well ossified and has well defined margins resulting in a distinctive cricoid bone. This character is thus identified as a synapomorphy supporting the molecular-based pairing of this clade. To date, morphological characters supporting the molecular identification of this clade [3, 7, 38] have remained elusive and thus, these findings are significant and provide phylogenetically informative data.

Cricoid cartilage- The cassowary cricoid FUR180 is characterised by a concave plate- or basin-like corpus, with a smooth dorsal and ventral surface. Neither the cassowary, emu, rhea, nor ostrich develop a median ridge traversing the dorsal (inner) surface of the main ventral plate/bowl of the cricoid [26 p. 73, 46, 48]. This crista is present in the kiwi [26 p. 72], moa and tinamous, projecting dorsally into the laryngeal lumen. Some moa species have two ridges of varying heights [58, 45 p. 106], similar to the cricoid of *N. maculosa* which has two slightly-raised ridges. The inverse of these ridges are recognisable on the ventral surface of the cricoid in both taxa (Figure 3E, F). The lateral margins of the Moa are smooth (figure 3F); this is also noted within the tinamou species *E. elegans*, although varying from the scalloped margins of the cricoid in *N. maculosa* (figure 3E). A small medially located cartilaginous caudal projection has been

observed in the rhea (figure 3C). This projection is often fused with tracheal cartilages and, in some species, is situated between two smaller, caudo-medially directed extensions [48]. Many moa genera, including *Dinornis* and *Pachyornis*, also develop these features [43, 58]. Caudal projections are absent in the cassowary. The emu and ostrich develop a rostral process [22, 26 p. 73, 46] which ossifies in the ostrich, acting as an attachment point for the cartilaginous basiurohyal.

Cartilaginous cricoid 'wings' extend seamlessly dorsocaudally from the lateral margins of the cricoid cartilage in the cassowary, ostrich, emu, and rhea. As no moa cricoid wings have been identified, despite the collection of multiple cricoid bones from fossil deposits, it is likely this element was also cartilaginous in moa. Ossification of the cricoid in moa [43, 45 p. 106] and tinamou (observations herein), indicates that the wings fused to the lateral borders of the cricoid were cartilagenous. This hypothesis is supported by images of a tinamou cricoid (Marcos Cenizo, Museo de Historia Natural de La Pampa). In all palaeognath taxa, the cricoid wings narrow dorsally and are directed caudally [26 p. 73, 46, 48]. In the tinamou, rhea [48], emu and ostrich [22, 26 p. 73, 46], the cricoid wings join dorsally, completing the cricoid ring caudal to the procricoid. In the cassowary, the wings do not articulate dorsally; instead the procricoid and a cranial projection from the medial point on the dorsal side of the second tracheal cartilage insert between the wing extremities.

Cart. procricoidea- The cassowary procricoid cartilage is formed of a flattened rectangular corpus with a distal, cranially extending head, and a proximal, caudally directed tail. The tail is triangular, with the cricoid-procricoid joints on the flattened dorsal edge. The head is rounded cranially and flattened laterally, forming the dorso-medial walls of the concave basins which run ventrally along both sides of the procricoid. This concavity supports the arytenoids which extend rostro-laterally from the procricoid. Both the procricoid and paired crico-procricoid joints are seemingly caudo-medially supported by the cranial extension of the second tracheal cartilage. Dorsally, the emu procricoid is a simple rectangular shape [26 p. 73], whereas the shape is wide and rounded rostrally in the ostrich. The ostrich procricoid also develops a ventro-caudally directed projection which extends between the cricoid wings [22]. The rhea procricoid is similar in shape although more angled, with a flattened rostral margin, and dorsally triangular caudal projection [48]. The dorso-cranial procricoid head, seen in the cassowary, is possibly absent in other palaeognaths, as is the cranial projection from the tracheal cartilages which sits below the procricoid. However, the literature provides no information on the procricoid for the tinamou, elephant bird or moa, with the descriptions for ostrich, rhea, and emu procricoids, brief and lacking detail.

Cart. arytenoidea- In the cassowary, the arytenoid corpus is cartilaginous, flat and elongated, extending laterally and ventrally to form two sides of a V-shape. The caudal end faces medially towards the opposing arytenoid and rests within the lateral procricoid joint concavities. Through the corpus and rostral projection, the flattened sides twist laterally to face dorsally. This arytenoid structure is shared with the emu, the closest relative of the cassowary although they vary with the lateral margins of the emu arytenoid converging rostrally into a cranio-medial point [26 p. 73]. The description of the arytenoid cartilage in the moa [43] suggests a similar structure, varying primarily in that the moa arytenoids are partly ossified. However, in the few preserved moa arytenoids in the NMNZ show more extreme curvature

throughout the bone. In contrast, the arytenoids of the rhea are formed from elongated, paired bars with proximally directed projections extending from the caudo-dorsal aspect of the arytenoids for attachment to the procricoid [48]. The ostrich arytenoids are also formed of elongate, paired bars, although have thin cartilaginous plates extending from the lateral margins, unique among palaeognaths. The plates form two lateral, and one dorsal projection with smooth, rounded margins.

Glottis- The arytenoids are covered in a dense mucosa, which forms the glottis mound. The dorsal surface of the glottis mound is typically smooth in palaeognaths including the cassowary; the only exception being the tinamous [26 p. 70]. Prominent laryngeal papillae extend from a widened caudal margin of the glottis mound in the rhea [48, 57, and kiwi [54]; the shape of the caudal papillae vary with angular, rounded, and rectangular forms (54). The lateral and dorsal projections of the arytenoid in the ostrich support the mucosal embellishments of the glottis mound, forming what has been termed a star-shape [22, 27, 49]. The lips of the glottis in both the ostrich and the rhea are supported internally by the arytenoids [22, 27, 48], however the glottis lips of the cassowary and emu [27] are not supported by the arytenoids but instead are formed of a separate mucosal structure, with a layer of dense musculature between.

Morphological Character Optimisation

To assess the phylogenetic signal of syringeal, hyoidal, and laryngeal (SHL) characters, we compared their fit to morphology-only, molecular-only, and combined data trees (see methods for full details). A fair comparison of character fit across trees is difficult due to radically different taxon sampling: notably most fossil taxa are missing from the molecular-only trees. However, the most important difference in the topologies concern relationships between major clades of ratites. Hence, we used the topology of a combined morphological and molecular data analysis, newly performed here, as one tree for comparison; to generate the other trees, we then re-arranged the major palaeognath groups to conform to either the morphology-only tree [59], or the molecular-only tree [3]. *Lithornis* was not sampled for the molecular tree and so thus left in its basal position as per the combined tree; however, this taxon is not codable for most SHL characters and so has very little impact on results (see supplementary data for results of phylogenetic analyses and character optimisation). For each topology, overall fit, as well as apomorphic and homoplasious states were identified. The results displayed in table 1 show the syrinx, hyoid and larynx characters to have a higher affinity for the combined-data topology than either the molecular- or morphological-only topologies. The tree length is lower for the combined-data topology (102), with results also showing a higher consistency index (CI= 0.5196) and lower character homoplasy (HI= 0.4804), both leading to a higher retention index (RI= 0.6818), the proportion of taxa with non-homoplasious states. The number of unique and unreversed apomorphic characters (CI = 1.0) is no less than 10 for the three topologies, although the data again favours the phylogenetic relationships obtained from the combined-data (AC = 14). Optimisation results for the morphological topology support the SHL data to have the lowest affinity for the morphological phylogenetic relationships.

Table 1:

Optimisation of SHL palaeognath characters on morphological, molecular and combined-data topologies. Tree index statistics for each optimised topology, including CI (consistency index), HI (homoplasy index), and RI (retention index), as well as AC (unique and unreversed apomorphic characters, CI = 1.0).

Topology	Tree Length	CI	HI	RI	AC
Morphological	108	0.4907	0.5093	0.6429	10
Molecular	106	0.5000	0.5000	0.6558	11
Combined Data (Mor + Mol)	102	0.5196	0.4804	0.6818	14

Figure 4:

Syrinx, hyoid, and larynx character optimisation onto morphology-only, molecular-only, and combined-data topologies. Characters identified include those optimised as unique and unreversed, unambiguous characters (black filled circles), homoplasious unambiguous characters (empty black circles), and unique and unreversed, ambiguous characters (grey filled circles). **A**, Morphological topology optimisation. **B**, Molecular topology optimisation. **C**, Combined-data topology optimisation.

In the following discussion, **ambiguous** changes are those which are optimisation-dependent (e.g. vary across deltran or acctran), and **unique and unreversed characters** are those with a CI of 1. We discuss and present (Figure 4) deltran results, but flag the optimisation-dependent changes as ambiguous. The three topologies show similar results for (homoplasious) autapomorphic character changes defining cassowaries. Three autapomorphic character changes are identified for all topologies: character 5, 2 –> 0 (bronchosyringeal cartilages wider at medial ends); character 6, 0 –> 1 (minor asymmetry present); character 11, 0 –> 1 (caudal end of the trachea almost cylindrical). All these changes have ambiguous optimisation due to missing data from *Casuarius bennetti*, (these changes might define the *C. bennetti* plus *C. casuarius* clade or *C. casuarius* alone). In all three phylogenies, 5 character changes consistently united both the cassowary and emu lineages: character 17, 1 –> 0 (cartilaginous basiurohyal); character 26, 1 ==> 2 (Numerous lingual papillae along the lateral margins of the tongue corpus); character 37, 1 –> 2 (cartilaginous arytenoids); character 40, 0 –> 1 (Arytenoid cartilage a separate structure to the glottis

lips); character 41, 1 ==> 0 (No laryngeal papillae present on the glottis lips). The changes for characters 26 and 40 are unique and unreversed for all three topologies.

As a result of missing data for the elephant bird syrinx, hyoid and larynx, there are no characters supporting the kiwi and elephant bird clade retrieved in the combined-data and molecular data topologies. Additionally, the character states identified as apomorphic for the kiwi are optimisation ambiguous as they might apply to the kiwi/elephant bird clade. In all three topologies, one of these characters is unique and unreversed for the kiwi: character 24, state change 0 to 1 (reduced ovular, tongue shape, dorsal view).

The tinamou-moa clade, robustly supported by DNA data but not predicted by traditional (skeletal) morphological trait, has new support from the SHL traits. This includes the ossification the cricoid cartilage (character 29, state 2) and the arytenoids (character 37, state 0). The character 37 change is an unambiguous optimised synapomorphy in both the molecular and combined-data topologies; this change is not unique and unreversed however as while unique within palaeognaths, it occurs outside the group, in sampled neognaths (*Grus, Gallus, Anseranas*). The state change for character 29 is optimised similarly for the molecular topology, however due to the basal placement of the tinamou and moa in the combined-data topology, a lack of complete cricoid ossification is optimised as an unambiguous synapomorphy for the crown palaeognaths, excluding the tinamou/moa clade. The functional significance of these ossifications is not obvious. Partial ossification of the cricoid has been recognised in older birds in taxa such as the long-legged buzzard [60], although ossification in the moa and tinamou is complete and consistent, with the trachea and syrinx of the moa also ossifying in all taxa.

Although rare among palaeognaths, ossification of the larynx, tracheal, and syrinx is common among Aves [61]. Ossification of the syrinx has been related to conferring rigidity in the syrinx as an adaptation for vocalisation, although the benefit of laryngeal and tracheal ossification remains unresolved. In comparison to mammalian counterparts, the avian trachea is well adapted to reducing chances of collapse with the presence of complete rings, and ossification is potentially a supplementary adaptation to assist with this [61]. If ossification is not an ancestral avian character linking tinamou and moa and other outgroup taxa, but a derived character, further investigation into the morphology of moa and tinamou may find drivers in call-type or behaviour related to the ossified state.

In addition, a cranial character, character 43 (state 1, articulation of the maxillary process of the nasal with the maxilla) was identified as an apomorphic character for the tinamou and moa through a review of the literature [62]. In the molecular topology, state 1 is placed unambiguously as a synapomorphy of the tinamou/moa clade; however, the character is not free of homoplasy as state 1 also occurs in *Lithornis* and the outgroups. In the combined data topologies, state 1 is again shared by the tinamou and moa, but not identified as synapomorphic due to the basal position of this clade within palaeognaths. Similarly to character 29 and 37 discussed above, because state 1 occurs in the sampled outgroup taxa (and also *Lithornis*) it is instead inferred to as the plesiomorphic state for palaeognaths, retained in tinamou and moa.

The identified link between discussed characters in the tinamou/moa clade and many outgroup taxa drove the syrinx, hyoid, and larynx data to support a tinamou/moa divergence more basal than that proposed in the molecular data topology. Although this is likely a result of characters retained from the last common ancestor between palaeognaths and neognaths, and not an indication of true descent, a recent study testing phylogenomic supertree methodologies on the avian topology, placed the tinamou/moa clade low in the palaeognath lineage [63] as the sister group to palaeognaths other than *Struthio*. The supertree was composed using three palaeognath phylogenetic analyses and three topological backbones, all of which prioritised molecular and genomic data and thus, morphological convergence should have had limited influence. Although no confirmation can be made, these results may indicate the divergence of tinamou and moa to be as of yet, unresolved relative to rheids in the tree.

In the molecular topology the basal positioning of the rhea and ostrich is supported by the unambiguous, unique and unreversed SHL character 35 (state 3, diamond shaped procricoid, dorsal view) and homoplasious, ambiguously optimised characters 10 (state 1, absence of the tympanum) and 39 (state 1, flattened arytenoid shape) identified as apomorphic for all other palaeognaths. Conversely, in the morphological topology, characters 35 (state 3) and 39 (state 1) optimise as unambiguous, unique and unreversed character states for the rhea and ostrich clade.

Discussion

Functional Morphology of the Syrinx, Hyoid, and Larynx in Palaeognaths

Syrinx- In comparison to most neognaths and other palaeognaths, the syrinx of the cassowary is poorly developed, is simple in structure, lacking elements such as the tympanum, lateral tympaniform membranes, and intrinsic musculature [18, 21]. The implications of this are poorly understood due to the location of the syrinx deep within the body, restricting research into how it functions [64]. Clarity of its functions is also hampered by the state of the ancestral syrinx remaining unknown, likely due to cartilaginous syringeal components being uncommon elements in the fossil record [35]. In spite of this, the simplicity of the cassowary syrinx in comparison to songbirds and the rhea, which produce a broader range of vocalisations, indicate support for the hypothesised direct relationship between anatomical complexity and vocal virtuosity [66].

The presence of structures such as the lateral tympaniform membrane and intrinsic musculature have previously been correlated with a broad vocal repertoire. For example, the presence of the lateral tympaniform membrane in the tinamou has been linked to the whistle-like notes, flute-like trill and alert or disturbed peeping calls they make [39]. Intrinsic musculature, arising and inserting within the syrinx, directly acts upon the syrinx to increase control over syringeal elements and allow for a greater sound vocabulary [65 – 67]. The functional significance of syringeal intrinsic musculature can observed in the kiwi and rhea, as both taxa likely utilise these muscles when vocalising. Rhea chicks develop broad repertoires, consisting of around five vocalisation types. Although this diminishes with age, vocalisation acts as the primary form of communication in chicks as it is better suited to the ecological contingencies

experienced during juvenile life stages, and thus, intrinsic muscles and increased syringeal complexity reflect this requirement [68]. Kiwi rely on sexually dimorphic calls to communicate over large distances during their nocturnally active periods and have the ability to produce a variety of distinctive calls [69].

A pessulus supports the syringeal membranes at the point of tracheal bifurcation, reducing chances of dorso-ventral collapse [17]. Although the pessulus is characteristic of the tracheo-bronchial syrinx and exists both in passerine and non-passerine taxa, its absence is not rare [19 p. 141, 34, 60]; many species in Psittacidae, and Columbidae [19 p. 141] lack a pessulus, as does the cassowary. Originally the pessulus and associated semilunar membrane were assumed to play some role in sound production, although its experimental removal resulted in no noticeable modification to sound production, suggesting structural support to be the only functional benefit of developing a pessulus [70, 71]. These findings indicate that increased functional support may be required when the syrinx is used for a broad range of vocalisations. Certainly, its absence in the cassowary and emu is correlated with a sparse vocal repertoire [18], and its presence in rhea [18] with complex vocalisations- at least in young. This does not explain however, the absence of the pessulus in the tinamou [39] and kiwi [18, 21], both of which have more complex vocalisations than rheas.

Absent in the cassowary, ossification of the syrinx is common among neognaths, enhancing strength and resistance of syringeal structures during vocalisation, when the potential muscular force applied is at its peak intensity. Although ossified elements are hollowed to reduce weight, presence of internal trabeculae maintain strength and reduce the likelihood of buckling or breakage [34]. Ossification may also allow for increased frequency – production of more rapid song elements – with efficient modulation and precise temporal control [34]. Such rapid movement and strength of syringeal elements is not required for the basic vocalisations produced by cassowaries, and therefore ossification of syringeal elements is absent. Ossification of syringeal elements occurs in moa and, alongside the presence of the pessulus, may indicate a resulting strengthening of the syrinx, thus suggesting a broader vocal repertoire, as in kiwi and rhea, than a cassowary.

Hyoid- Morphological similarity of hyoid elements between the cassowary and emu was expected due to their well-established close relationship. Despite this, the hyoid apparatus in the cassowary and emu differ, primarily in relative length of structures; i.e. the cassowary ceratobranchials are elongate compared to the epibranchials, while in the emu both structures have similar length. This is likely a reflection of the different feeding strategies between the two taxa, with studies finding evidence for a strong functional relationship between feeding mode and cranio-lingual morphology [72, 73]. Other than the frugivorous cassowary, all other palaeognaths have a varied diet, comprised of leaves, flowers, fruits and grass seeds, as well as dicot herbs and shrubs [74]. Often various insects and small animals are also included in the diets of most palaeognaths, particularly the kiwi, primarily feeding on soil invertebrates [75 - 77].

Elongation of hyoid elements in Aves is directly associated with tongue protrusion and its increased control, with extreme morphologies noted for the taxa such as woodpeckers and hummingbirds [23 p. 53, 78]. Of the palaeognaths for which the hyoid apparatus has been assessed, the tinamou has the most

elongate hyoid elements comparative to basiurohyal length, although the ceratobranchials of the cassowary are similarly elongate. The cassowary employs the ‘catch and throw’ feeding method; a method of feeding which requires a reduced tongue so as not to get injured during the feeding process [28, 79]. For this method there is no requirement for tongue protrusion, an action impossible for the cassowary with its small tongue. Therefore, it is likely that minor elongation of the ceratobranchials may be associated with control of the tongue, allowing the cassowary to arrange the tongue in a position that doesn’t inhibit food manipulation.

Extensive ossification of avian hyoid elements has previously been noted within both the paired ceratobranchials and epibranchials, as well as in the midline elements, the basihyal and urohyal, and has been linked to the attachment of muscles associated with coordination of hyoid movement primarily during feeding [47]. Nonetheless, no association between basiurohyal ossification and diet within palaeognaths has been identified. The cassowary and ostrich basiurohyals are cartilaginous, whereas ossification is present in tinamou and moa, with no obvious link to diet. The tinamou and ostrich are omnivores, the moa was an herbivore and the cassowary is a specialist frugivore [52]. Therefore, it is likely that hyoid element ossification in palaeognaths is not directly related to the diet of each taxon and may instead be more directly associated with evolutionary relationships and the diets of ancestral taxa. Ossification of the ceratobranchials and lack of ossification in the epibranchials is relatively consistent among all taxa [23 p. 52, 24 p. 362, 80], although unexpectedly ossification of epibranchials occurs within the tinamou. Research into the functional implication of this is incomplete with limitations concerning variation between closely related taxa where the diet is similar [72].

Larynx- The avian larynx has previously received little attention, with the functional purpose and significance of the morphology and ossification of elements only briefly reported. Analyses which assess the structure and functional significance of the larynx in Aves primarily focus on the associated musculature which, although functionally important, provide little insight into the functional role of the skeletal morphology. The ontogenetic rate of ossification in the larynx differs by individual element and by species, with findings suggesting a heavy influence from the size of the species and age of the individual [25, 26 p. 74]. However, without analysis of numerous individuals or complex ontogenetic studies, the effect of age on ossification cannot be determined. Aside from age, ossification of laryngeal elements such as the cricoid of the moa and tinamou has been explained by Hogg [61] as a supplementary modification to increase support against collapse. Hogg [61] did concede however that determining the necessity of ossification in the trachea and larynx was problematic, and no comparison was made between his study taxa, domestic fowl, and taxa with similar ecologies which lack an ossified larynx. Various differences in the feeding ecologies and body size of the moa and tinamou do not assist in determining the functional significance of ossification of elements within clade.

Morphological Support for Palaeognath Molecular Phylogeny

The results of the optimisation of syringeal, hyoidal and laryngeal characters onto the morphological, molecular and combined-data topologies suggests these structures might provide novel phylogenetic information. The results outlined in Table 1 show the morphological data for the syrinx, hyoid and larynx structures in palaeognaths had the highest concordance for the combined-data topology, followed by the molecular topology. Interestingly, the morphological topology was the least-preferred.

As no syrinx, hyoid or larynx elements have been recovered for the any aepyornithids, the results of the study shed no new morphological light on the molecular kiwi plus elephant-bird clade. However, apomorphic character states currently known only in the kiwi may be apomorphic for the kiwi plus elephant bird clade. To test this, such structures would need to be recovered for the elephant bird. This is unlikely however: the cartilaginous state of these structures in the kiwi suggest they are also cartilaginous in elephant birds, which is also consistent with their lack of appearance in the fossil record. If these elements were consistently and completely ossified, they might have been preserved, as in the moa.

The new SHL characters shared by the tinamou and moa provides novel morphological support for the sister placement of these two taxa in molecular topologies. This is important as morphological apomorphic characters for this clade have been elusive. Additionally, a cranial character not included in previous phylogenetic analyses, is newly recognised as a synapomorphy for the moa/tinamou clade: presence of the articulation of the maxillary process of the nasal with the maxilla [see 62].

Methods

Specimen

The cassowary analysed in this study was an adult female acquired from the Department of Environment and Heritage Protection, Innisfail, Queensland. Death occurred on June 9th, 2016, when the cassowary was hit by a vehicle, south of Tully, Queensland. The complete ossification and synostosis of all compound elements including the skull, presence of fully developed ovaries, and a weight of over 40 kg led to the conclusion that the cassowary was a fully mature adult female. The frozen cassowary was transported to Flinders University on permit number I13689, issued 16th January 2017. Its skeleton is catalogued FUR180in the Flinders University Palaeontology Collection.

Scanning and Modelling

Following the methodology of Clement et al. [81], the hyoid apparatus, larynx and syrinx were extracted as a single element from the cassowary body, and dehydrated in increasingly strong concentrations of ethanol (C_2H_6O), 70% and 85%, prior to being placed in an iodine, ethanol solution formed from 2L of 100% ethanol and 200g of iodine (Iodine ACS reagent >99.8 solid). The contrast agent iodine was selected for its differential affinities to the major soft tissue types, and safety of use [82 - 84]. Contrast agents differentially stain soft tissue types making visible increased contrast between tissues enabling

higher levels of detail of morphology, organisation, and arrangement to be captured when CT or μCT scanned [84 - 86].

The specimen was μCT scanned on the 11th May 2018 at Adelaide Microscopy (the University of Adelaide). A 2006 Skyscan-1076 in vivo x-ray microtomograph (Bruker Micro CT, Kontich, Belgium) machine was used to scan the specimen, with a resultant pixel size of 35 microns. The resulting image collections from the full scans were amalgamated into volume images [82]. 3D modelling and segmentation of the μCT volume images was conducted through thresholding and segmentation in Mimics [87] to produce clean, reliable 3D models of skeletal elements. Elements were modelled and edited individually, prior to being reconstructed as a single colume.

Description and Comparative Analysis

Comparative anatomy examination of the cassowary specimen was completed based on interpretation of elements from the literature, primarily syrinx and larynx descriptions from King [19] and McLelland [26] respectively, with nomenclature derived from Baumel *et al.* [88]. Comparative species morphologies were also derived from the literature, athough information was lacking for some palaeognaths, primarily kiwi, moa and tinamou, and a complete absence noted for elephant birds. Data on the cricoid of the kiwi was obtained from Catherine Tate, Dr Jean-Claude Stahl, and Alan Tennyson of the Museum of New Zealand Te Papa Tongarewa. Laryngeal and hyoid element data for two tinamou species was also obtained from Marcus Cenizo of the Museo de Historia Natural de La Pampa. All available moa specimens in the Museum of New Zealand Te Papa Tongarewa, wherein presence of tracheal ring sets predicated the possible presence of syringeal and laryngeal structures, were examined by PM.

Phylogenetics

Morphological Characters and Molecular Backbone

42 characters (SI. 2.) were coded in Mesquite [89] for 22 palaeognath species representing eight families and six outgroup taxa. Outgroup species were coded from available literature, as well as reference specimens. This includes the crane, *Grus rubicunda*; coded for all non-SHL morphological characters by Trevor Worthy. Palaeognath and outgroup species from Worthy and Scofield [59] were selected to allow comparison with previous analyses and for incorporation into future morphological analyses. An additional character concerning the articulation of the maxillary process of the nasal bar with the maxilla was developed from Mayr [62] and also included, along with a molecular dataset from Grealy *et al.* [3]. Morphological characters were ordered, with ordering of skeletal characters from Mitchell *et al.* [38].

Phylogenetic Analyses

We used the complete dataset, both morphological and molecular to assess the phylogenetic utility of the syringeal, hyoidal, and laryngeal structures discussed above. We modified the dataset over three analyses to produce morphological, molecular and combined topologies on which the syrinx, hyoid and larynx characters could be optimised.

The morphological topology was produced through parsimony phylogenetic analyses conducted in PAUP* [90] and implemented through CIPRES Scientific Gateway (91, version 3.3). Taxon with no morphological data were excluded from the analyses which was run with the heuristic algorithm and 5000 random addition replicates per search, using tree-bisection-reconnection (TBR) branch-swapping. The stepwise-addition option selected was random. Maximum likelihood bootstrap analysis [92] was also utilised to estimate nodal support using the heuristic search methods with 500 replicates. Trees were rooted by the 6 outgroup taxa, all characters were weighted equally.

Bayesian methods produced the molecular and complete combined-data topology, selected for as management of missing data is more reliable in Bayesian methods than parsimony [93, 94]. Topologies were inferred through the Markov Chain Monte Carlo (MCMC) and the maximum-likelihood methods, statistical procedures used in Bayesian analysis, in the program MrBayes (95, version 3.2.2), and implemented through the CIPRES Scientific Gateway (91, version 3.3). Bayesian inference was run with a burn in fraction of 25% for 30 million generations, sampling every 10 thousand and employing four chains (one cold and three incrementally heated). The analysis was run using the PartitionFinder best fit substitution model for each subset, with the temperature of the MCMC analysis set to 0.08. Molecular subset branch lengths were linked and scaled by substitution rate. Other default parameters were used and molecular backbone constraints were implemented.

Character optimisation

The three above phylogenies provide hypotheses which can be tested against the new syrinx, hyoid and larynx (SHL) data. Optimisation of SHL characters were performed using parsimony methods in PAUP, under both accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN); the fit of the SHL characters against each tree was addressed using total tree length, as well as ensemble Consistency and Retention indices. Unambiguous and unique and unreversed character were identified as important for their contribution to defining individual clades.

Abbreviations

FU- Flinders University, Bedford Park (5042), South Australia, Australia

MMC- Marcos Cenizo Personal Collection, Santa Rosa (6300), La Pampa, Argentina

NMNZ- Museum of New Zealand Te Papa Tongarewa, Wellington (6011), New Zealand

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing Interests

The authors declare they have no competing interests.

Funding

No funding was required for this study.

Author Contributions

THW obtained the permits for the specimen. PLM and THW conceived the research and AMC and PLM iodine stained and scanned the specimen. PLM segmented, 3D modelled and carried out the morphological, comparative and phylogenetic analyses on the specimen as well as drafting the manuscript with guidance from all authors. All authors edited and approved the final manuscript.

Acknowledgements

We thank Alan Tennyson, Jean Claude Stahl and Catherine Tate from the Museum of New Zealand Te Papa Tongarewa (Wellington, New Zealand) for assisting wither the collection of data and proving photographs of reference specimens. Also, Marcos Cenizo from Museo de Historia Natural de La Pampa (Santa Rosa, Argentina) for their time sorting and dissecting tinamou specimens, and for taking photographs of required structures.

References

1. Hackett SJ, Kimball RT, Reddy S, Bowie RCK, Braun EL, Braun MJ, et al. A phylogenomic study of birds reveals their evolutionary history. Science. 2008;320:1763-1768.
2. Harshman J, Braun EL, Braun MJ, Huddleston CJ, Bowie RCK, Chojnowski JL, et al. Phylogenomic evidence for multiple losses of flight in ratite birds. PNAS. 2008;105:13462-13467.
3. Grealy A, Phillips M, Miller G, Gilbert MTP, Rouillard JM, Lambert D, et al. Eggshell palaeogenomics: Palaeognath evolutionary history revealed through ancient nuclear and mitochondrial DNA from

Madagascan elephant bird (*Aepyornis*) eggshell. Molecular Phylogenetics and Evolution. 2017;109:151-163.

4. Sibley CG, Ahlquist JE. Phylogeny and classification of birds. New Haven: Yale University Press; 1990.
5. Phillips MJ, Gibb GC, Crimp EA, Penny D. Tinamous and Moa flock together: Mitochondrial genome sequence analysis reveals independent losses of flight among ratites. Systematic Biology. 2010;59:90-107.
6. Johnston P. New morphological evidence supports congruent phylogenies and Gondwana vicariance for palaeognathous birds. Zoological Journal of the Linnean Society. 2011;163:959-982.
7. Yonezawa T, Segawa T, Mori H, Campos PF, Hongoh Y, Endo H, et al. Phylogenomics and morphology of extinct palaeognaths reveal the origin and evolution of the ratites. Current Biology. 2017;27:68-77.
8. Dickinson EC, Remsen JV, editors. The Howard & Moore complete checklist of the birds of the world. 4th EastBourne: Aves Press; 2013.
9. Davies SJJF. Cassowaries. In: Davies SJJF. Ratites and Tinamous: Tinamidae, Rheidae, Dromaiidae, Casuariidae, Apterygidae, Struthionidae. New York: Oxford University Press; 2002. p. 228-241.
10. Bradford MG, Westcott DA. Consequences of southern cassowary (*Casuarius,*) gut passage and deposition pattern on the germination of rainforest seeds. Austral Ecology. 2010;35:325-333.
11. Abourachid A, Renous S. Bipedal locomotion in ratites (Palaeognathiform): examples of cursorial birds. Ibis. 2000;142:538-549.
12. Naish D, Perron R. Structure and function of the cassowary's casque and its implications for cassowary history, biology, and evolution. Historical Biology. 2016;24:5-7-581.
13. Ryan M, Brenowitz E. The role of body size, phylogeny, and ambient noise in the evolution of bird song. The American Naturalist. 1985;126:87-100.
14. Podos J, Huber SK, Taft B. Bird song: the interface of evolution and mechanism. Annual Review of Ecology, Evolution and Systematics. 2004;35:55-87.
15. Podos J, Nowicki S. Beaks, adaptation, and vocal evolution in Darwin's finches. BioScience. 2004;54:501-510.
16. Mason NA, Burns KJ, Tobias JA, Claramunt S, Seddon N, Derryberry EP. Song evolution, speciation, and vocal learning in passerine birds. Evolution. 2016;71-3:786-796.
17. Cevik-Demirkan A, Haziroglu RM, Kurtul I. Gross morphological and histological features of larynx, trachea and syrinx in Japanese quail. Anatomia, Histologia, Embryologia. 2007;36:215-219.
18. Forbes WA. On the conformation of the thoracic end of the trachea in the ratite birds. Proceedings of the Zoological Society of London. 1881;778-788.
19. King AS. Functional anatomy of the syrinx. In: King AS McLelland, J, editors. Form and function in birds. London: Harcourt Brace Jovanovich Publishers; 1989. p. 69-103.
20. Beddard F. The structure and classification of birds. London: Longmans, Green and co.; 1898.

21. Pycraft W. On the morphology and phylogeny of the Palaeognathae (Ratite and Crypturi) and Neognathae (Carinate). Transactions of the Zoological Society of London. 1900;15:149-290.
22. Soley JT, Tivane C, Crole MR. Gross morphology and topographical relationships of the hyobranchial apparatus and laryngeal cartilages in the ostrich (*Struthio camelus*). Acta Zoologia. 2015;96:442-451.
23. Homberger DG. The avian lingual and laryngeal apparatus within the context of the head and jaw apparatus, with comparisons to the mammalian condition: Functional morphology and biomechanics of evaporative cooling, feeding, drinking, and vocalisation. In: Maina J, editor. The biology of the avian respiratory system: Evolution, development, structure and function. Switzerland: Springer; 2017. p. 27-97.
24. Tomlinson CAB. Feeding in Palaeognathous Birds. In: Schwenk K, editor. Feeding: form and function in tetrapod vertebrates. San Diago: Academic Press; 2000. p. 359- 394.
25. Zweers GA, van Pelt HC, Beckers A. Morphology and mechanics of the larynx of the pigeon (*Columba livia*): A drill-chuck system (Aves). Zoomorphology. 1981;99:37-69.
26. McLelland J. Larynx and Trachea. In: King AS, McLelland J, editors. Form and function in birds. London: Harcourt Brace Jovanovich Publishers; 1989. p. 69-103.
27. Crole MR, Soley, JT. What prevents *Struthio camelus* and *Dromaius novaehollandiae* (Palaeognathae) from choking? A novel anatomical mechanism in ratites, the linguo-laryngeal apparatus. Frontiers in Zoology. 2012;9:11-17.
28. Erdogan S, Iwasaki S. Function-related morphological characteristics and specialised structures of the avian tongue. Annals of Anatomy. 2014;196:75-87.
29. Crole MR, Soley JT. Morphology of the tongue of the emu (*Dromaius novaehollandiae*). I. Gross anatomical features and topography. Onderstepoort Journal of Veterinary research. 2009;76:335-345.
30. Mauricio GN, Areta JI, Bornschein MR, Reis RE. Morphology-based phylogenetic analysis and classification of the family Rhinocryptidae (Aves: Passeriformes). Zoological Journal of the Linnean Society. 2012;166:377-432.
31. Hughes JM. Monophyly and phylogeny of cuckoos (Aves, Cuculidae) inferred from osteological characters. Zoological Journal of the Linnean Society. 2000;130:263-307.
32. Livezey BC, Zusi RL. Higher-order phylogeny of modern birds (Theropoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion. Zoological journal of the Linnean Society. 2007;149:1-95.
33. Manegold A. Morphological characters of the tongue skeleton reveal phylogenetic relationships within the Corvidae (Oscines, Passeriformes). Emu- Austral Ornithology. 2008;108:321-330.
34. During DN, Ziegler A, Thompson CK, Ziegler A, Faber C, Muller J, et al. The songbird syrinx morpheme: a three-dimensional, high-resolution, interactive morphological map of the zebra finch vocal organ. BMC Journal of Biology. 2013;11:1-27.

35. Clarke JA, Chatterjee S, Li Z, Riede T, Agnolin F, Goller F, et al. Fossil evidence of the avian vocal organ from the Mesozoic. Nature. 2016;538:502-505.
36. Parker WK. VII. On the structure and development of the skull in the ostrich tribe. Philosophical Transactions of the Royal Society. 1866;156:113-183.
37. Livezey BC. A phylogenetic analysis of the Gruiformes (Aves) based on morphological characters, with an emphasis on the rails (Rallidae). Philosophical Transactions of the Royal Society of London B. 1998;353:2077-2151.
38. Mitchell KJ, Llamas B, Soubrier J, Rawlence NJ, Worthy TH, Wood J, et al. Ancient DNA reveals elephant bird and kiwi are sister taxa and clarifies ratite bird evolution. Science. 2014;344:898-900.
39. Garitano-Zavala A. Evolutionary loss of the extrinsic syringeal muscle sternotrachealis in Darwin's Nothura (*Nothura darwinii*) - Pérdida evolutiva del músculo siríngeo extrínseco esternotraqueal en *Nothura darwinii*. The Auk. 2009;126:134-140.
40. Fowler ME. Comparative clinical anatomy of ratites. Journal of Wildlife Medicine. 1991;22:204-227.
41. Yildiz H, Bahadir A, Akkoc A. A study on the morphological structure of syrinx in ostriches (*Struthio camelus*). Anatomia, Histologia, Embryologia. 2003;32:187-191.
42. Picasso MBJ, Carril J. The peculiar syrinx of *Rhea americana* (greater rhea, Palaeognathae). Vertebrate Zoology. 2013;63:321-327.
43. Oliver WRB. The Moas of New Zealand and Australia. Wellington: Dominion Museum Bulletin; 1949.
44. Owen, R . Brain, larynx, and trachea in the genus Dinornis. Transactions of the Zoological Society. 1871;7:385-392.
45. Worthy TH, Holdaway RN. Prehistoric life of New Zealand: the lost world of the moa. Indiana: Indiana University Press; 2002.
46. Tadjalli M, Mansouri SH, Poostpasand A. Gross anatomy of the oropharyngeal cavity in the ostrich (*Struthio camelus*). Iranian Journal of Veterinary Research 2008;9:316-323.
47. Li Z, Zhou Z, Clarke JA. Convergent evolution of a mobile bony tongue in flighted dinosaurs and pterosaurs. PLOS One. 2018;13:e0198078.
48. Crole MR, Soley JT. Gross anatomical features of the tongue, lingual skeleton and laryngeal mound of *Rhea americana* (Palaeognathae, Aves): morpho-functional considerations. Zoomorphology. 2012;131:265-273.
49. Faraggiana R. Sulla morfologia della lingua e del rialzo laringeo di alcune specie di Ucc. Bollettino dei Musei di Zoologia ed Anatomia Comparata Della. 1933;43:131-323.
50. Jackowiak H, Ludwig M. Light and scanning electron microscopic study of the structure of the ostrich (*Struthio camelus*) tongue. Zoological Science. 2008;25:188-194.
51. Maxwell EE. Comparative ossification and development of the skull in palaeognathous birds (Aves: Palaeognathae). Zoological Journal of the Linnean Society. 2009;156:184-200.
52. Bradford MG, Westcott DA. Predation of cassowary dispersed seeds: is the cassowary an effective disperser?. Integrative Zoology 2011;6:168-177.

53. Crole MR. Soley JT. Gross morphology of the intra-oral rhamphotheca, oropharynx and proximal oesophagus of the emu (*Dromaius novaehollandiae*). Anatomia Histologia Embryologia. 2010;39:207-218.
54. McCann C. The tongues of kiwis (*Apteryx*). Notornis. 1973;20:123-127.
55. Sales J. Feeding of the captive kiwi. Zoos' Print Journal. 2006;21:2454-2458.
56. Cunningham SJ, Corfield JR, Iwaniuk AN, Castro I, Alley MR, Birkhead TR, et al. The anatomy of the bill tip of kiwi and associated somatosensory regions of the brain: comparisons with shorebirds. PLOS One. 2013;8:e80036.
57. Rodrigues MN, Tivane CN, Carvalho RC, Olivera GB, Silva RSB, Ambrosio CE, et al. Gross morphology of the rhea oropharyngeal cavity. Pesquisa Veterinaria Brasileira. 2012;32:53-59.
58. Scarlett R. Thyroid bones of moas. Records of the Canterbury Museum. 1975;9:247-258.
59. Worthy TH, Scofield RP. Twenty-first century advances in knowledge of the biology of the moa (Aves: Dinornithiformes): a new morphological analysis and moa diagnosis revised. New Zealand Journal of Zoology. 2012;39:87-753.
60. Kabak M, Orhan IO, Haziroglu RM. The anatomy of larynx, trachea and syrinx in the Long-legged buzzard (*Buteo rufinus*). Anatomia, Histologia, Embryologia. 2007;36:27-32.
61. Hogg DA. Ossification of the laryngeal, trachea and syringeal cartilages in the domestic fowl. Journal of Anatomy. 1982;134:57-71.
62. Mayr G. Comparative morphology of the avian maxillary bone (os maxillare) based on an examination of macerated juvenile skeletons. Acta Zoologica. 2018; doi: 10.1111/azo.12268.
63. Kimball RT, Oliveros CH, Wang N, White ND, Barker FK et al. A phylogenomic supertree of birds. Diversity. 2019; doi: 10.3390/d11070109.
64. Elemans CP, Muller M, Larson ON, van Leeuwen JL. Amplitude and frequency modulation control of sound production in a mechanical model of the avian syrinx. The Journal of Experimental Biology. 2009;212:1212-1224.
65. Greenwalt C. Bird song: Acoustics and physiology. Washington: Smithsonian Institution Press; 1968.
66. Gaunt AS. An hypothesis concerning the relationship of syringeal structure to vocal abilities. The Auk. 1983;100:853-862.
67. Gaunt AS, Gaunt SL. Electromyographic studies of the syrinx in parrots (Aves, Psittacidae). Zoomorphology. 1985;105:1-11.
68. Beaver PW. Ontogeny of vocalisation in the Greater Rhea. The Auk. 1978;95:382-388.
69. Corfield J, Gillman L, Parsons S. Vocalisations of the North Island Brown Kiwi (*Apteryx Mantelli*). The Auk. 2008;125:326-335.
70. Miskimen M. Sound production in passerine birds. The Auk. 1951;68:493-504.
71. Gaunt AS, Wells MK. Models of syringeal mechanisms. American Zoologist. 1973;13:1227-1247.
72. Gardner LL. The adaptive modifications and the taxonomic value of the tongue in birds. Proceedings of the United States National Museum. 1925;67:1-49.

73. Li Z, Clarke JA. The craniolingual morphology of waterfowl (Aves, Anseriformes) and its relationship with feeding mode revealed through contrast-enhanced X-ray computed tomography and 2D morphometrics. Evolutionary Biology. 2016;43:12-25.
74. Wood JR, Rawlence NJ, Rogers GM, Austin JJ, Worthy TH, Cooper A. Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves, Dinornithiformes). Quaternary Science Reviews. 2008;27:2593-2602.
75. Marchant S, Higgins PJ. Handbook of Australian, New Zealand & Antarctic Birds. Volume 1. Part A: Ratites to Petrels. Melbourne: Oxford University Press; 1990.
76. Noble JC. On ratites and their interactions with plants. Revista Chilena de Historia Natural. 1991;64:85-188.
77. Mosa SG. Fall and winter diet and habitat preferences of the Andean tinamou (*Nothura pentlandii*) in the northwest Argentina. Studies on Neotropical Fauna and Environment. 1993;28:123-128.
78. Jung JY, Naleway SE, Yaraghi N, Herrera S, Sherman V, Bushong EA, et al. Structural analysis of the tongue and hyoid apparatus in a woodpecker. Acta Biomaterialia. 2016;37:1-13.
79. Erdogan S, Alan A. Gross anatomical and scanning electron microscopic studies of the oropharyngeal cavity in the European magpie (*Pica pica*) and the common raven (*Corvus corax*). Microscopy Research and Technique. 2012;75:379-387.
80. Korzun LP Érard C, Gasc J. Morphofunctional study of the bill and hyoid apparatus of *Momotus momota* (Aves, Coraciiformes, Momotidae): implications for omnivorous feeding adaptation in motmots. Biologie et Pathologie Animales. 2004;327:319-333.
81. Clement AM, Nysjö J, Strand R, Ahlberg PE. Brain – endocast relationship in the Australian lungfish, *Neoceratodus forsteri*, elucidated from tomographic data (Sarcopterygii: Dipnoi). PloS One. 2015;10:e0141277.
82. Metscher BD. MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralised animal tissues. BMC Physiology. 2009;9:11-25.
83. Degenhardt K, Wright AC, Horng D, Padmanabhan A, Epstein JA. Rapid 3D phenotyping of cardiovascular development in mouse embryos by micro-CT with iodine staining. Circulation: Cardiovascular Staining. 2010;3:314-322.
84. Gignac PM, Kley NJ, Clarke JA, Colbert MW, Morhardt AC, Cerio D, et al. Diffusible iodine-based contrast-enhanced computed tomography (diceCT): an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. Journal of Anatomy. 2016;228:889-909.
85. Gignac PM, Kley NJ. Iodine-enhanced micro-CT imaging: methodological refinements for the study of the soft-tissue anatomy of post-embryonic vertebrates. Journal of Experimental Zoology. 2014;322B:166-176.
86. Thorn C, Sedlmayr J, McNulty M. Utilising iodine to image alligator soft tissue structures with Micro-CT. The FASEB Journal. 2015;29:suppl. 1.
87. Mimics version 18.0, 3D medical image processing software. Belgian; Materialise NV: 2017.

88. Baumel JJ, King AS, Breazile JE, Evans HE, Vanden Berge JC, editors. Handbook of Avian Anatomy: Nomina Anatomica Avium. Massachusetts: Publications of the Nuttall Ornithological Club; 1993.
89. Maddison WP, Maddison DR. Mesquite: a modular system for evolutionary analysis. Version 3.40. http://www.mesquiteproject.org. 2018.
90. Swofford DL. PAUP*. Phylogenetic analysis using parsimony (*and other methods) version 4.0a. Massachusetts: Sinauer Associates; 2003.
91. Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. New Orleans: Proceedings of the Gateway Computing Environments Workshop (GCE); 2010. p. 1-8.
92. Felsenstein J. Confidence limits on phylogenies: an approach using bootstrap. Evolution. 1985;39:783-791.
93. Gatesy J, Matthee C, Desalle R, Hayashi C. Resolution of a supertree/supermatrix paradox. Systematic Biology. 2002;27:522-527.
94. Malia MJ, Lipscomb DL, Allard MW. The misleading effects of composite taxa in supermatrices. Molecular Phylogenetics and Evolution. 2003;27:522-527.
95. Ronquist F, Huelsenbeck JP. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003;20:1572-1574.

Supplementary Information

SI 1. Moa species presence and absence of tracheal rings (.docx)

A list of all moa taxa with recovered tracheal rings in the Museum of New Zealand Te Papa Tongarewa.

SI 2. Syrinx, hyoid, and larynx characters and character states (.docx)

All characters developed through morphological analysis of the syrinx, hyoid and larynx in palaeognaths, used in the phylogenetic analyses and optimised onto the three resulting topologies.

SI 3. Character coding for 28 palaeognath and outgroup taxa (.docx)

Character coding for all palaeognath and outgroup taxa assessed throughout this study.

SI 4. Video footage of 3-dimensional models for the syrinx, hyoid, and larynx of the Southern Cassowary (.avi)

Three individual short videos of the 3D models of the syrinx, hyoid, and larynx. The images turn on a single axis to show the structures from various angles.

SI 5. Input data for three phylogenetic analyses (.txt)

Two text files. One includes the complete input file with all included data for the combined phylogenetic analysis conducted using Bayesian methods. The second file includes the input for the two parsimony phylogenetic analyses, molecular and morphological. This file excludes the taxa character data which can be found in the combined-data analysis file.

SI 6. Input data for the optimisation character analyses

Complete input file for the optimisation analyses which were run in the program PAUP* [89], with coding for all three tested topologies.

SI 7. Optimisation Results

Results for optimisation analyses of SHL data onto the combined-data topology which produced better results than either the morphological- or molecular-only topologies.

All data will be deposited in dryad upon acceptance of this paper, and a reference to the data will be added to the reference list.

Figures

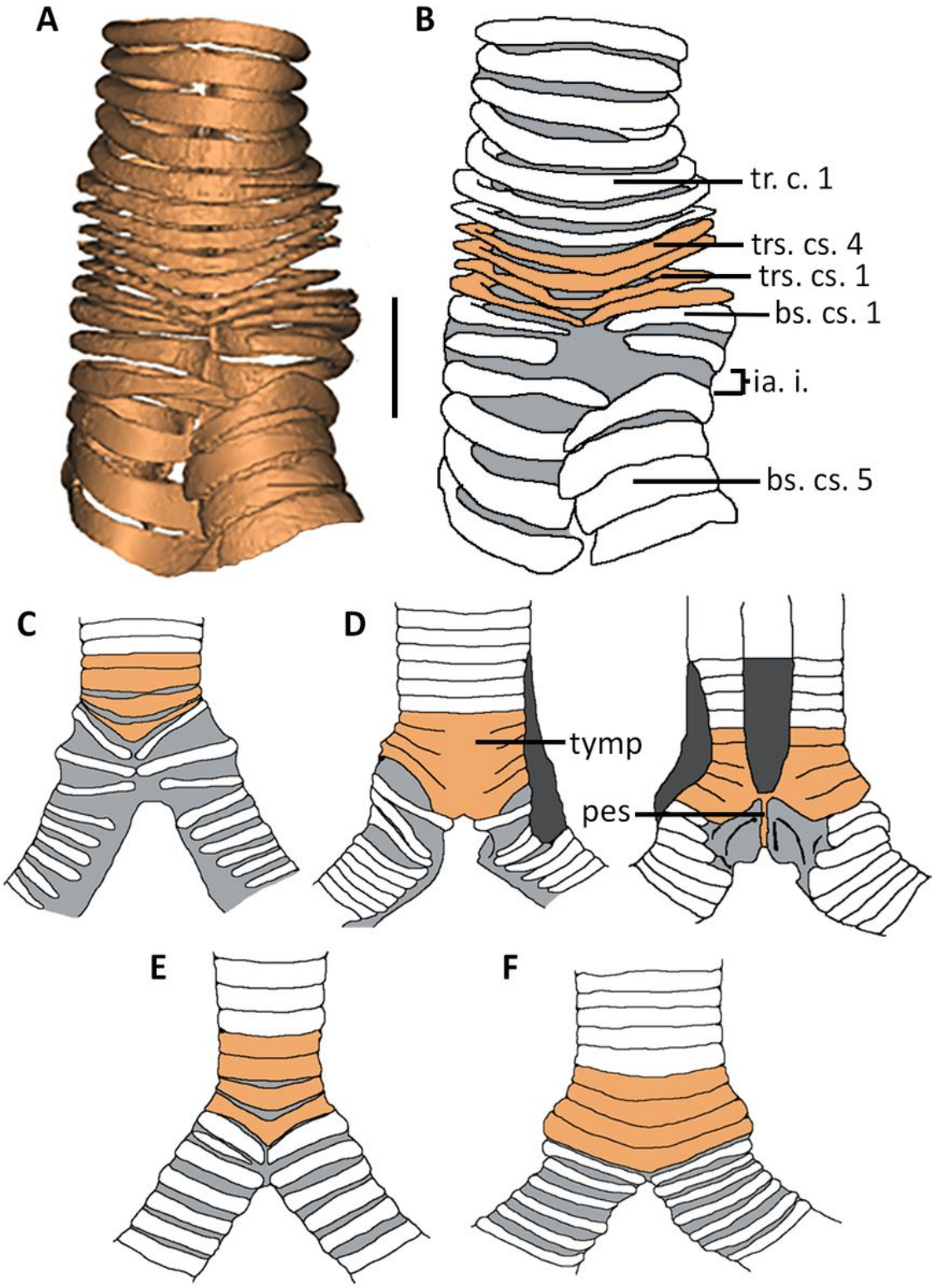

Figure 1

Palaeognath syringeal elements with tracheosyringeal cartilages differentiated by brown shading (B-E), interannular tissues are shaded in grey. A-B, Cassowary, Casuarius casuarius, FUR180; ventral, scale bar = 10mm. C, Tinamou, Nothura darwinii, adapted from Garitano-Zavala [39, fig. 1C]; ventral. D, Rhea, Rhea americana, adapted from Forbes [18, fig. 7, 8]; ventral and dorsal. E, Kiwi, Apteryx mantelli, adapted from Forbes [18, fig. 3], ventral. F, Ostrich, Struthio camelus, adapted from Forbes [18, fig. 1]; ventral.

Abbreviations: bs.cs: bronchosyringeal cartilages, ia.i: interannular interval, tr.c: tracheal cartilages, trs.cs: tracheosyringeal cartilages. C-F scaled to same size approximately.

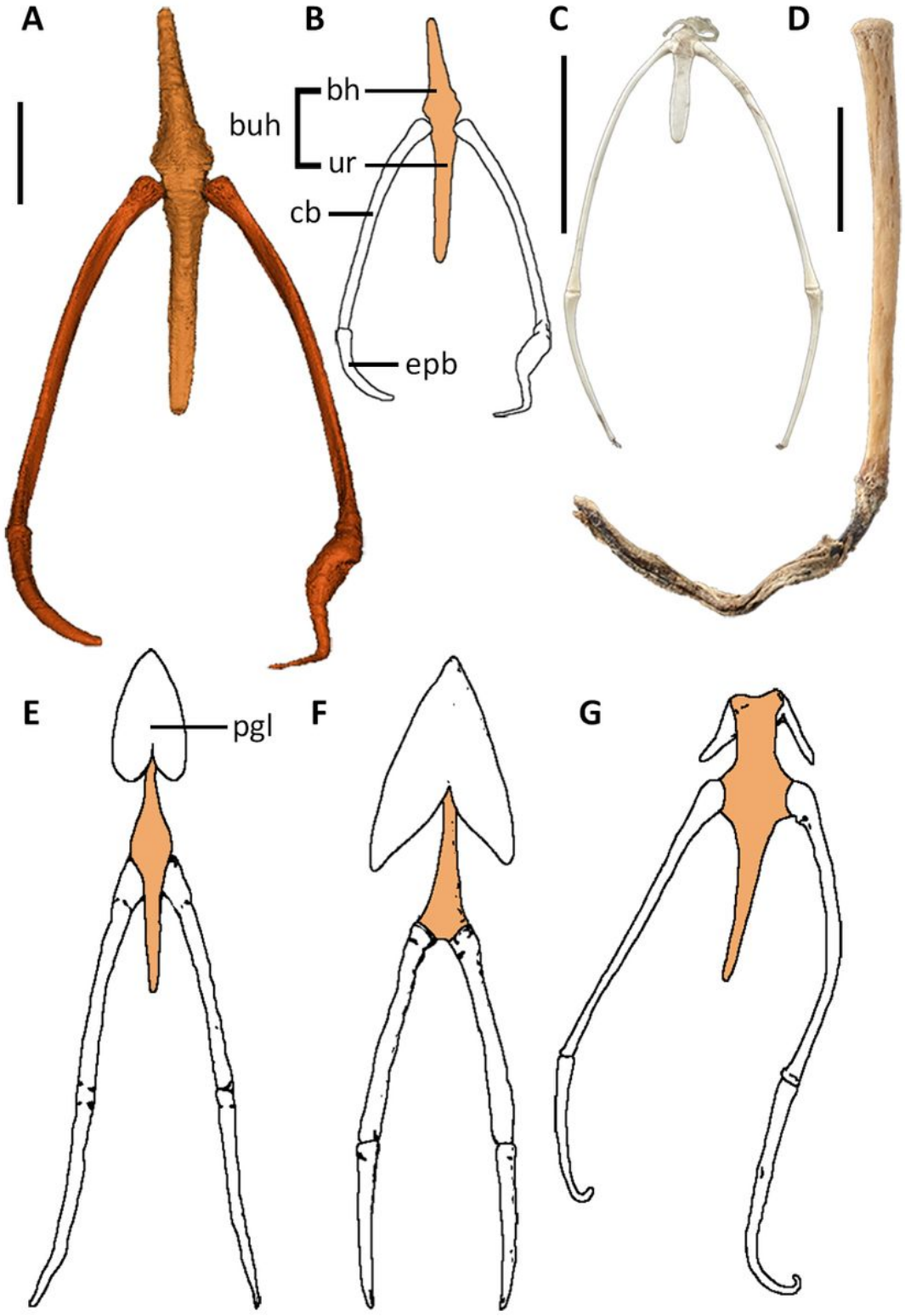

Figure 2

Palaeognath hyoid elements. A-B, Cassowary, Casuarius casuarius, FUR180; dorsal view, A- scale bar = 10mm. C, Tinamou, Nothoprocta perdicaria, NMNZ S. 22983; dorsal view, scale bar = 10mm. D, Moa, Megalapteryx, NMNZ S. 400; dorso-lateral view, scale bar = 10mm. E, Emu, Dromaius novaehollandiae,

adapted from Parker [36, plate XII]; dorsal view. F, Rhea, Rhea americana, adapted from Parker [36, plate X]; dorsal view. G, Ostrich, Struthio camelus, adapted from Soley et al. [22, fig. 3]; dorsal view. Abbreviations: bh: basihyal, buh, basiurohyal, cb: ceratobranchial, epb, epibranchial, ur: urohyal.

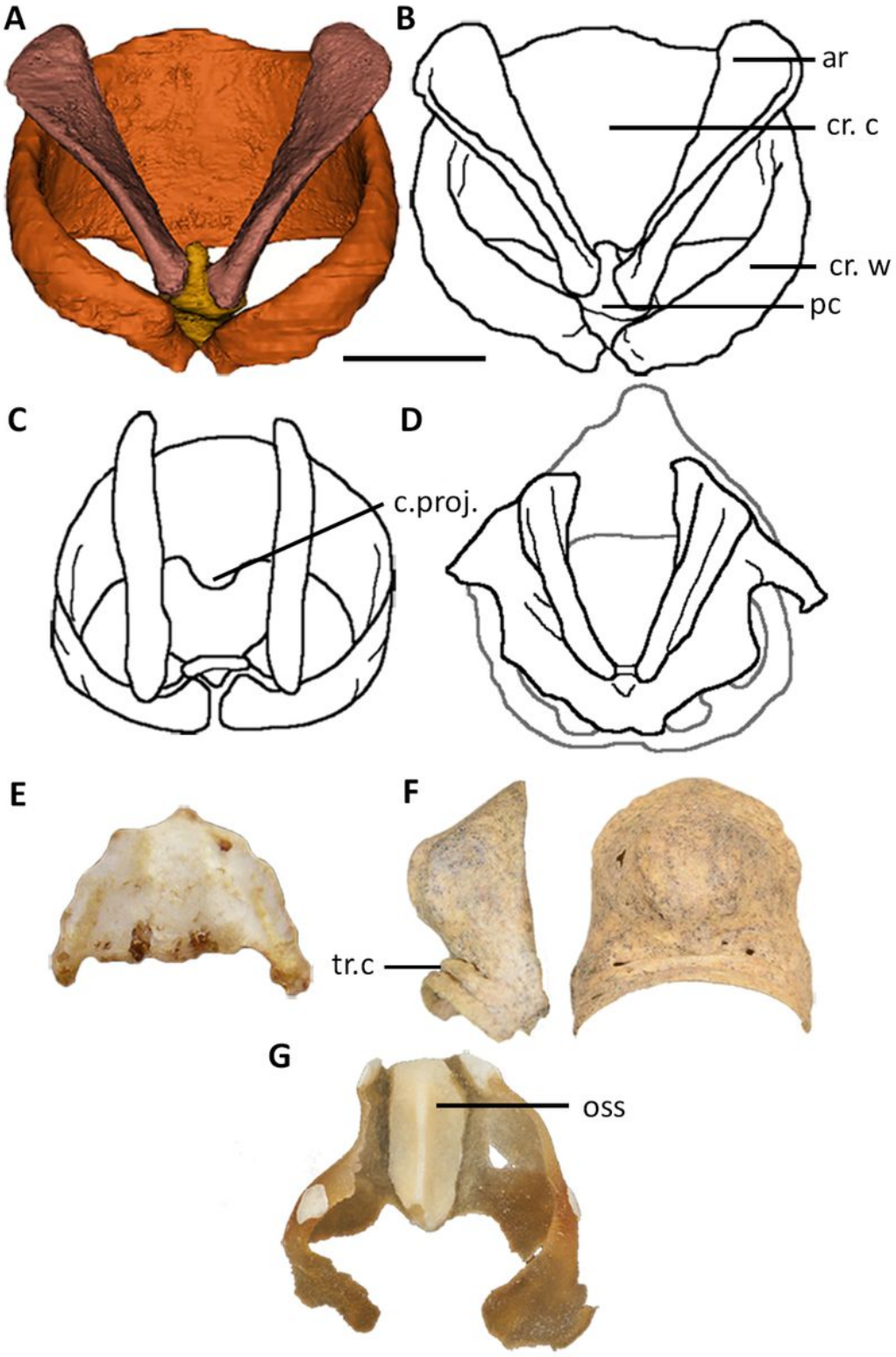

Figure 3

Palaeognath laryngeal elements. A-B, Cassowary, Casuarius casuarius, FUR180; dorsal view, scale bar = 10mm. C, Rhea, Rhea americana, adapted from Crole and Soley [48, fig. 7]; dorsal view. D, Ostrich, Struthio

camelus, adapted from Tadjalli [46, fig. 9a, b]; dorsal view. E, Tinamou, Nothura maculosa, MMC321; ventral view. F, Moa, Euryapteryx curtus, NMNZ S. 44757; lateral and ventral views. G, Kiwi, Apteryx rowi, NMNZ OR.27243A; dorsal view. Abbreviations: ar: arytenoids, cr.c: cricoid cartilage, cr.w: cricoid wings, oss: ossification, pc: procricoid, tr.c: tracheal cartilages.

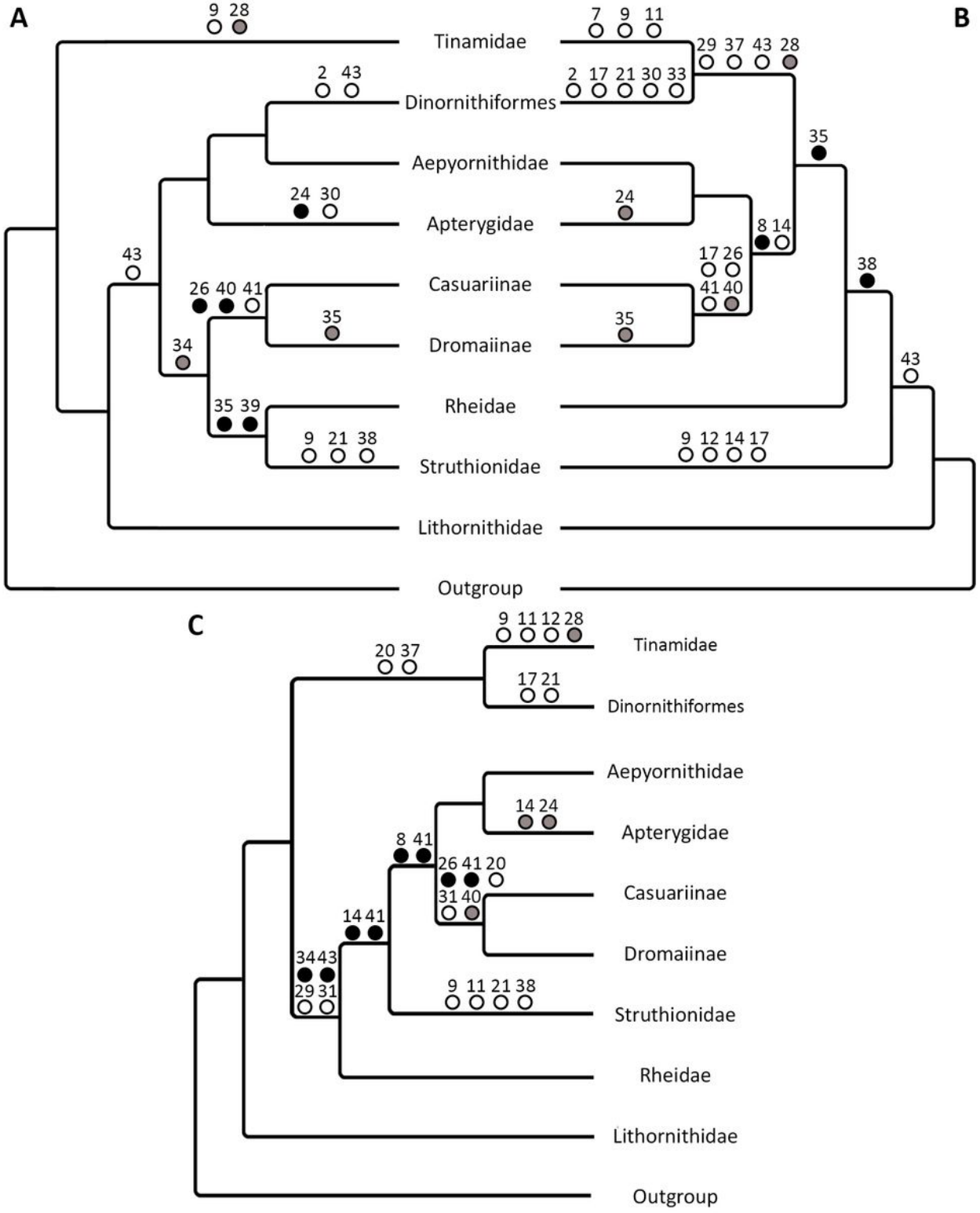

Figure 4

Syrinx, hyoid, and larynx character optimisation onto morphology-only, molecular-only, and combined-data topologies. Characters identified include those optimised as unique and unreversed, unambiguous characters (black filled circles), homoplasious unambiguous characters (empty black circles), and unique and unreversed, ambiguous characters (grey filled circles). A, Morphological topology optimisation. B, Molecular topology optimisation. C, Combined-data topology optimisation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.txt
- supplement2.txt
- supplement3.pdf
- supplement4.txt
- supplement5.txt
- supplement6.avi
- supplement7.avi
- supplement8.avi