Figure S1

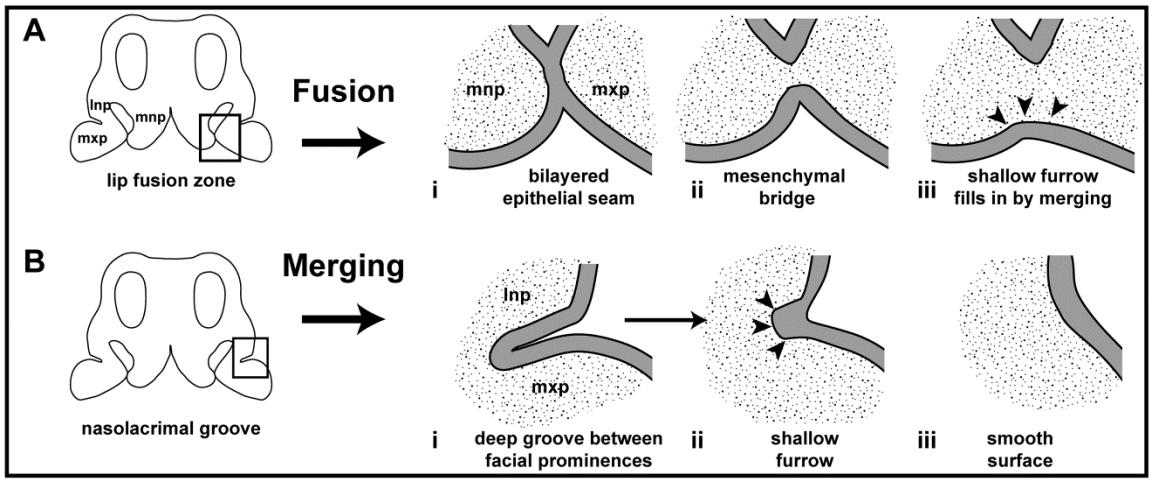

Figure S1

Schematic depicting the processes of fusion and merging in the primary palate. A) Fusion occurs between two separate prominences which grow towards each other and fuse. A bilayered epithelial seam forms (i), followed by a mesenchymal bridge (ii) and then finally the external furrows are filled in by merging (iii, black arrowheads). B) Merging occurs between the lateral nasal and maxillary prominences. Initially there is a deep groove (i) which becomes shallower (ii) via differential proliferation and/or possibly cell migration (black arrowheads). At last the external surface becomes completely smoothed out (iii).

Figure S2

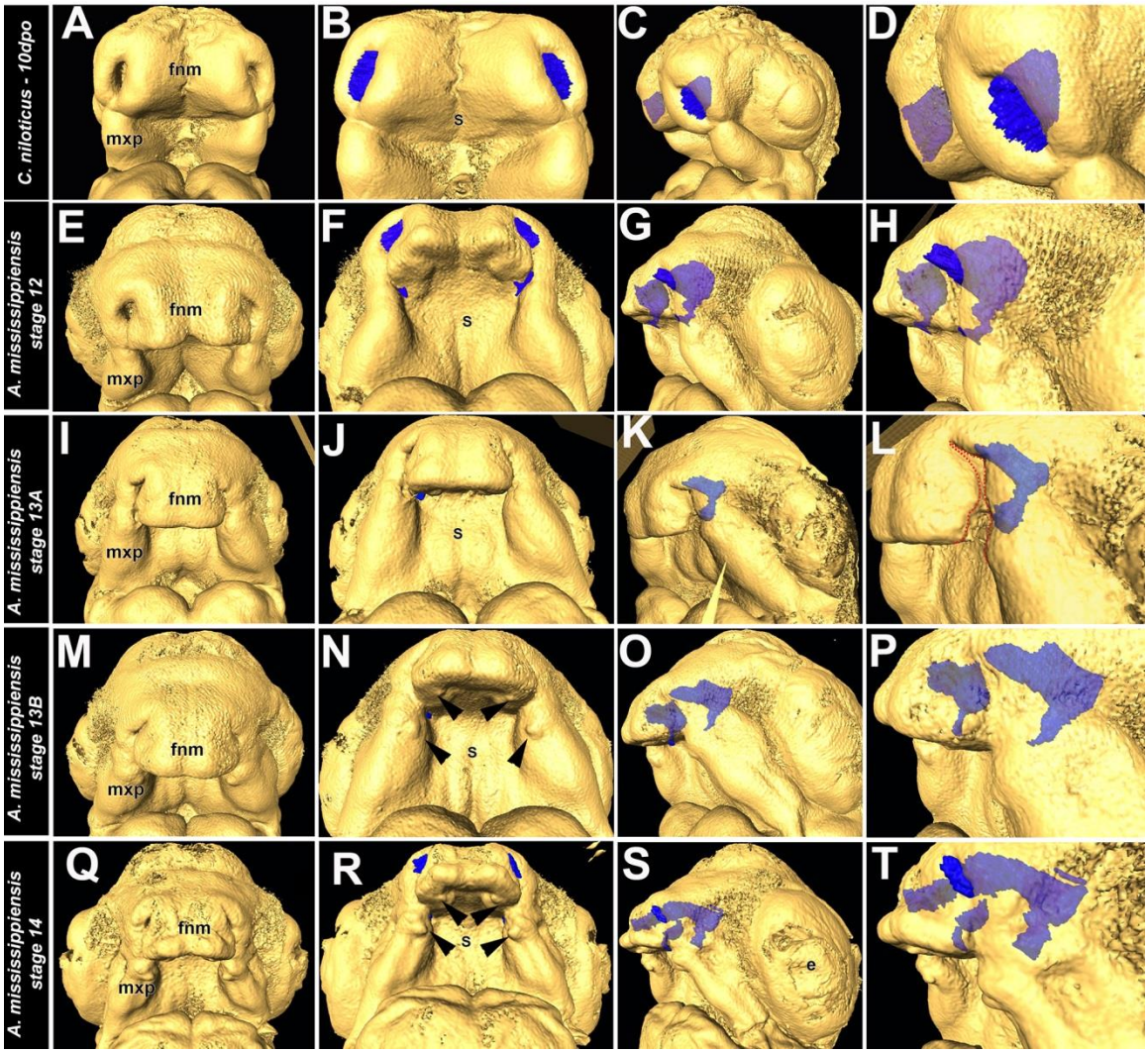

Figure S2

Crocodilian choana formation. A-D) OPT reconstructions of 10-dpo crocodile. The nasal pits are widely separated and there is a deep furrow in the centre of the frontonasal mass. B-D) There is a blunt end to the nasal passages. E-H) Stage 12 alligator. Judging from the increased size of the eyes, projection of the frontonasal mass from the head, the decreased distance between the nasal slits and lengthening of the maxillary prominences this embryo is developmentally advanced compared to the crocodile. F-H) The choanae are open and connected to the external nares. I-L) Stage 13 alligator has a relatively narrow frontonasal mass and the midline furrow has smoothed out. The choana are open (J,K,L). M-P) A slightly older stage 13 embryo. Superficial, first generation teeth are visible in the maxillary prominences (black arrowheads) and the choanae are open. Q-T) The stage 14 alligator embryo has increased projection of the frontonasal mass frm the head. There are two superficial teeth in each maxillary prominence as well as teeth in the dorsal side of the frontonasal mass. Key: fnm, frontonasal mass; mxp, maxillary prominence; s, stomodeum.

Figure S3

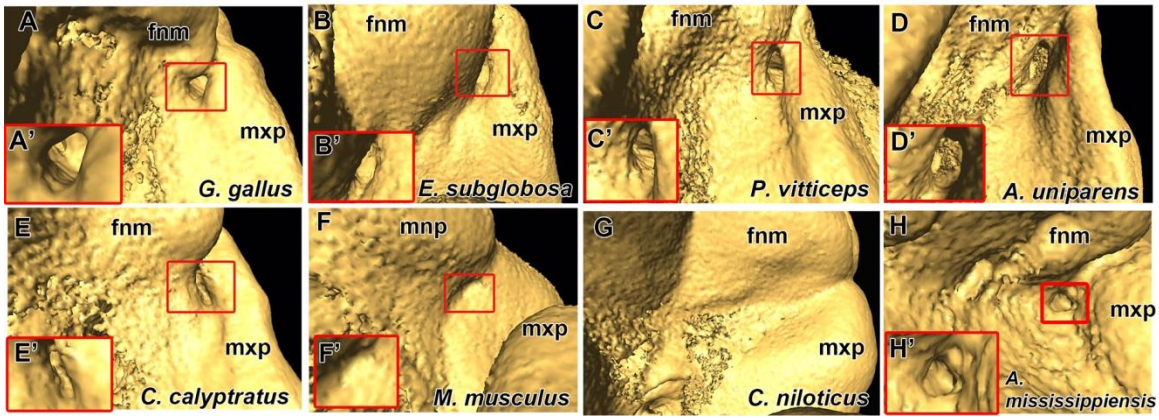

Figure S3

High power views of the choanae in chicken, non-avian reptiles and mammals. Inset images show magnified views of choanae in each panel. Key: fnm, frontonasal mass; mnp, medial nasal prominence; mxp, maxillary prominence.

Figure S4

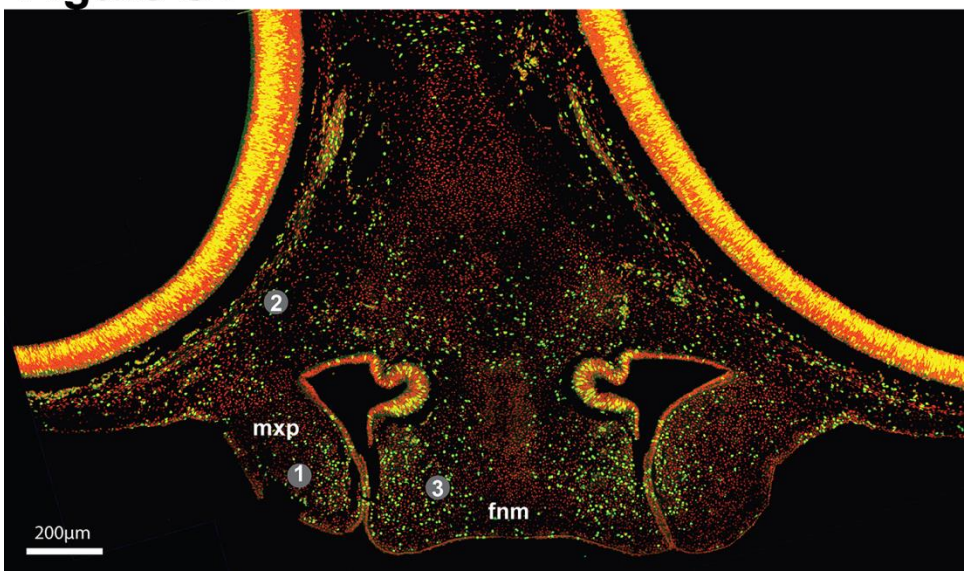

Figure S4

BrdU labeling of a stage 31 bearded dragon (*P. vitticeps*) visible in coronal section at similar level as in chicken in Figure 5. Regions 1 and 3 clearly exhibit higher proliferation than region 2, in a similar pattern to that of chicken.

Figure S5

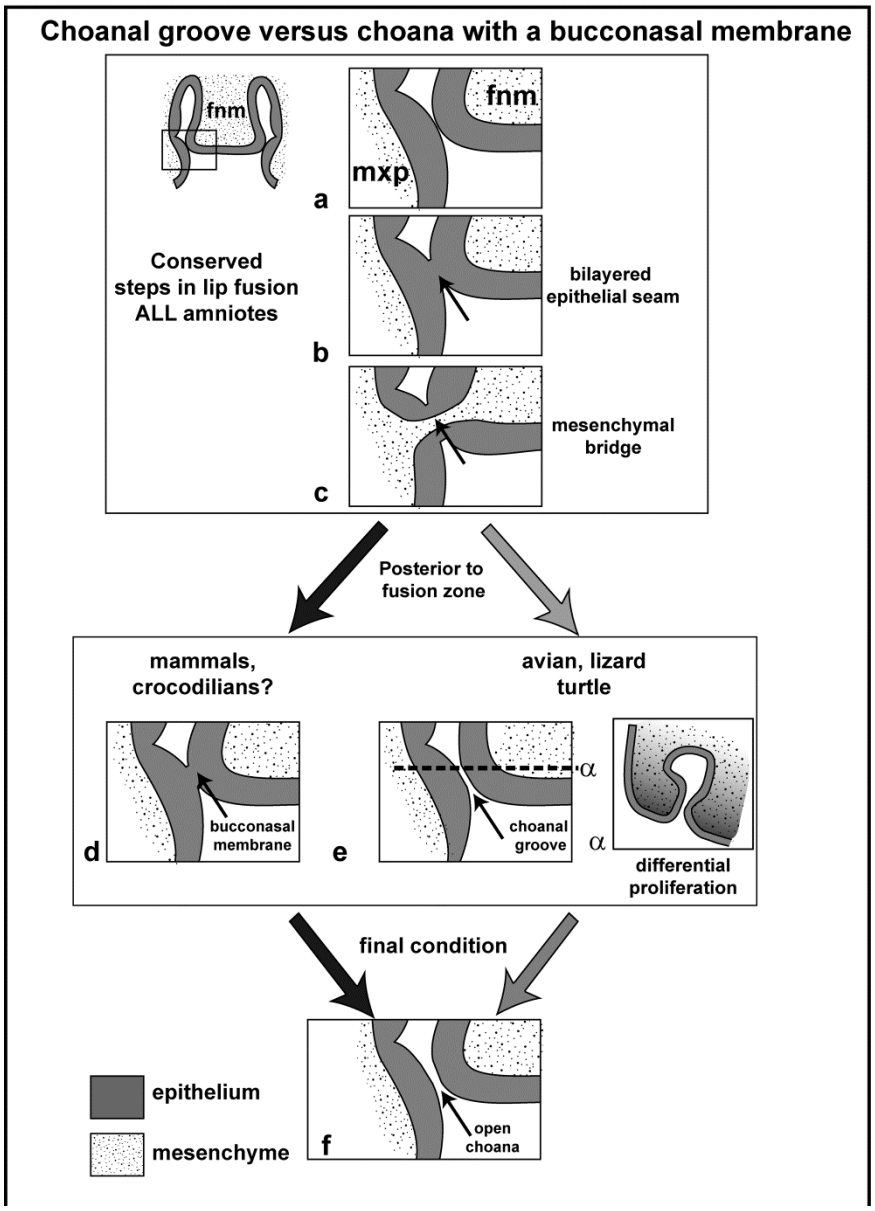

Figure S5

Schematic summarizing the conserved steps and branching points during choana formation. Steps a-c are conserved in all amniotes, although the actual prominences that initiate the fusion varies according to taxa. Steps d and e are alternative paths to forming the choana. In d, just posterior to the mesenchymal bridge, the epithelium persists forming the bucconasal membrane. In e, the epithelium is not present and an open groove is present. In the coronal plane (α), there is a decline in proliferation at the base of the choana (lighter grey). The final state (f) of all amniotes is to have an open choana connecting the oral and nasal cavities.

Supplementary Movie Legends

Supp. Movie 1

Fly through frontal sections of 15 dpo crocodile (*C. niloticus*) through the region of the nasal cavities. Approximately half-way through the fly through the lateral nasal and frontonasal prominences are fused and connected by a mesenchymal bridge. Superior to the fusion are the open nasal passages line with thickened olfactory epithelium. The last third of the fly through enters the maxillary prominences and at this level the nasal passages open into the oral cavity (choanae). Therefore the choanae are continuously open from the external nares to the stomodeum.

Supp. Movie 2

Rotational movie of a stage 27 chicken embryo nasal cavity prior to fusion. The choanal groove and nasal cavity can clearly be seen between the unfused prominences throughout the animation.

Supp. Movie 3

Rotational movie of a stage 28 chicken embryo just after initial fusion has occurred between the frontonasal mass and anterior maxillary prominence. A deep groove between the prominences still remains that will be filled in by merging. The open choana can be observed at the end of the animation.