1   # A crocodylian-style cloaca in a non-avialan dinosaur

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14

15   **Abstract**

16   Our knowledge of the reproductive biology of dinosaurs covers a range of aspects, from

17   brooding behaviour to nesting style and the timing of sexual maturity. Yet, the basic anatomy

18   and function of the cloaca in non-avialan dinosaurs remains unknown. Here, we describe the

19   outer morphology of the only known non-avialan dinosaur cloaca, preserved in an

20   exceptional specimen of the early-diverging ceratopsian dinosaur *Psittacosaurus*. We clarify

21   the position of the cloaca with respect to the ischia and caudal vertebrae and document the

22   scales immediately adjacent to the abdomen and tail. We find that the cloaca is from a near-

23   sexually mature subadult individual and is most similar to the cloaca of crocodylians, to the

24   exclusion of lepidosaurians and birds. However, the sex of SMF R 4970 could not be

25   determined as the cloaca and the rest of the specimen does not yield any sexually dimorphic

26   information. This study highlights the ongoing role of exceptional specimens in providing

27   rare soft tissues that help to bridge longstanding gaps in our knowledge of the basic biology

28   of dinosaurs and other extinct reptiles.

29

30   **Introduction**

31   The reproductive biology of extinct non-avialan dinosaurs is rarely interpreted from the fossil

32   record. To date, exceptionally well-preserved remains and the extant phylogenetic bracket

33   (EPB; [1]) have clarified details including their brooding behaviour, nesting style and timing

34   of sexual maturity [2-7]. However, the anatomy and function of the cloaca has continued to

35   remain elusive. In archosaurian and lepidosaurian reptiles, the cloaca forms the common

36   opening of the digestive and urogenital tract and consists of a series of chambers—the

37   coprodeum, urodeum, and proctodeum—separated by muscular sphincters, and which

38   terminates in the vent [8-10]. As a result, it serves as the passage for digestive and urinary

39   wastes, transmission of the male copulatory organ, and the passage of eggs or live young in

40   addition to a variety of less obvious functions such as salt and temperature regulation [11].

41   Nearly 70 years ago, Alfred Romer predicted that the cloaca in extinct archosaurs was

42   positioned within the proximal tail region where haemal arches are absent [1]. Here, we

43   provide confirmation of Romer's original prediction based on an exquisitely-preserved

44   specimen of an early-diverging ceratopsian dinosaur, *Psittacosaurus* sp., discovered from the

45   Early Cretaceous Jehol Group of northeastern China [12]. This specimen, known for its

46   exquisite coloured epidermal structures and elongate caudal monofilaments [12-14],

47   preserves for the first time the outer morphology of the cloaca in a non-avialan dinosaur.

48

49   **Results**

50   *Age of the individual*

51   The right femur of Senckenberg Museum Frankfurt (SMF) R 4970 is ~140mm long. This is

52   similar to the femoral lengths of *P. lujiatunensis* Institute of Vertebrate Paleontology and

53   Paleoanthropology (IVPP) V12617 (138mm) and V18344 (145mm), Liaoning

54   Paleontological Museum (LPM) R00128 (135mm) and R00138 (144mm) and Peking

55   University Vertebrate Paleontology (PKUVP) V1053 (149mm) and V1056 (135mm) which

56   belong to ~6-7 year old subadults (see Table 1 and Fig. 5 of Erickson *et al*. [15] and

57   Supplementary Table S2 of Zhao *et al*. [16]). This age is just shy of sexual maturity and at

58   the beginning of the exponential growth phase (see Table 1 and Fig. 5 of Erickson *et al*. [15]

59   and Supplementary Table S2 of Zhao *et al*. [16]). The femoral length of SMF R 4970 is

60   therefore the closest match to a nearly sexually mature subadult (see Table 1 and Fig. 5 of

61   Erickson *et al*. [15] and Supplementary Table S2 of Zhao *et al*. [16]). Thus, the configuration

62   and morphology of the cloaca in SMF R 4970 and its surrounding osteological correlates had

63   not yet reached its most developed form or full sexual maturity.

64

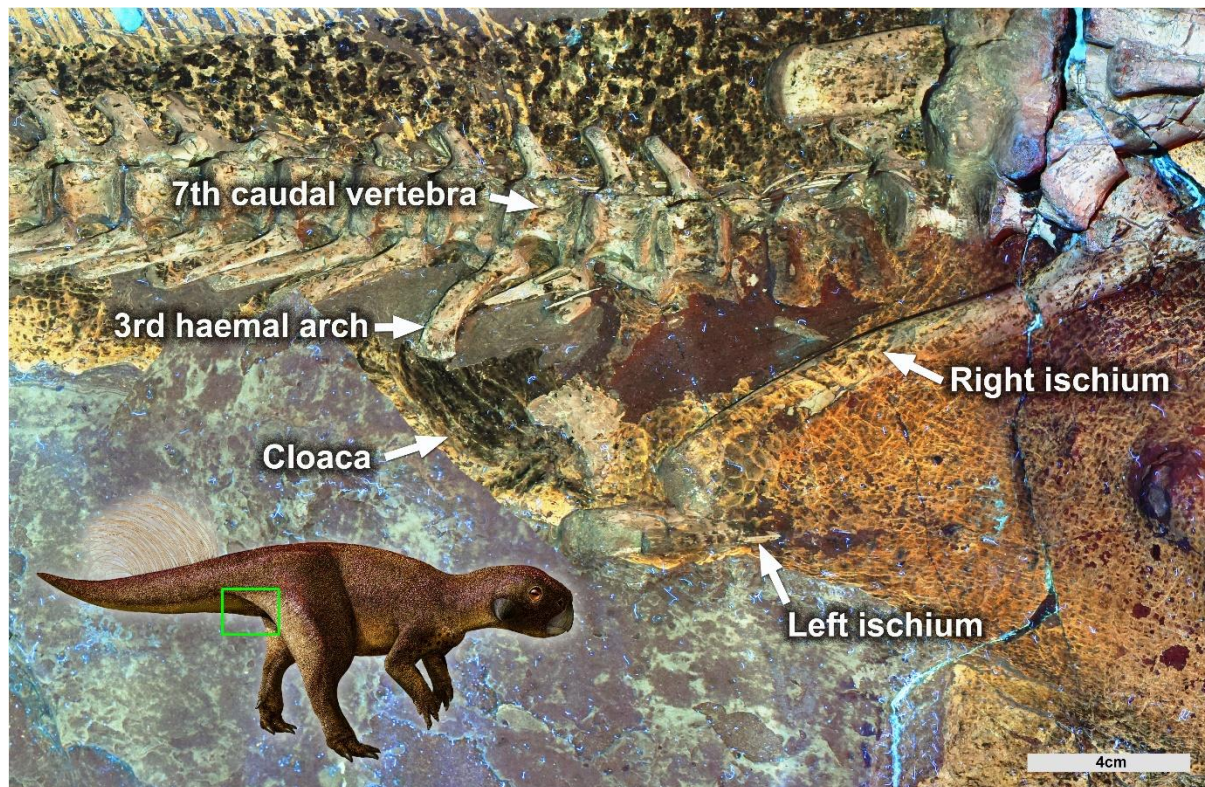65   *The cloaca and its surrounding scales and bones*

66

Figure 1. Cloacal region of the ceratopsian dinosaur *Psittacosaurus* under Laser-Stimulated Fluorescence (LSF). The cloaca soft tissue is the blackish mottled ovoid area preserved in the region between the ischia and the third haemal arch in specimen SMF R 4970 from the Early Cretaceous Jehol Group of northeastern China. Scale is 4cm. Life reconstruction with green box showing the position of the LSF image. Image Credit: Julius T. Csotonyi (image used with permission).

Immediately posterior to the ischiadic symphysis is the fleshy aperture (or vent) of the cloaca (Figs. 1, 2). Since SMF R 4970 is preserved lying on its back, both the left and right sides of the cloaca are visible, although the right side is better exposed (Fig. 1). The vent itself is longitudinally oriented (~2 cm long) and surrounded on either side by darkly pigmented tissue. This appears to lie flush with the ventral parts of the abdomen and tail (i.e. it does not form a cloacal protuberance). The darker cloaca is clearly differentiated, from the lighter-coloured surrounding integument (Fig. 2).
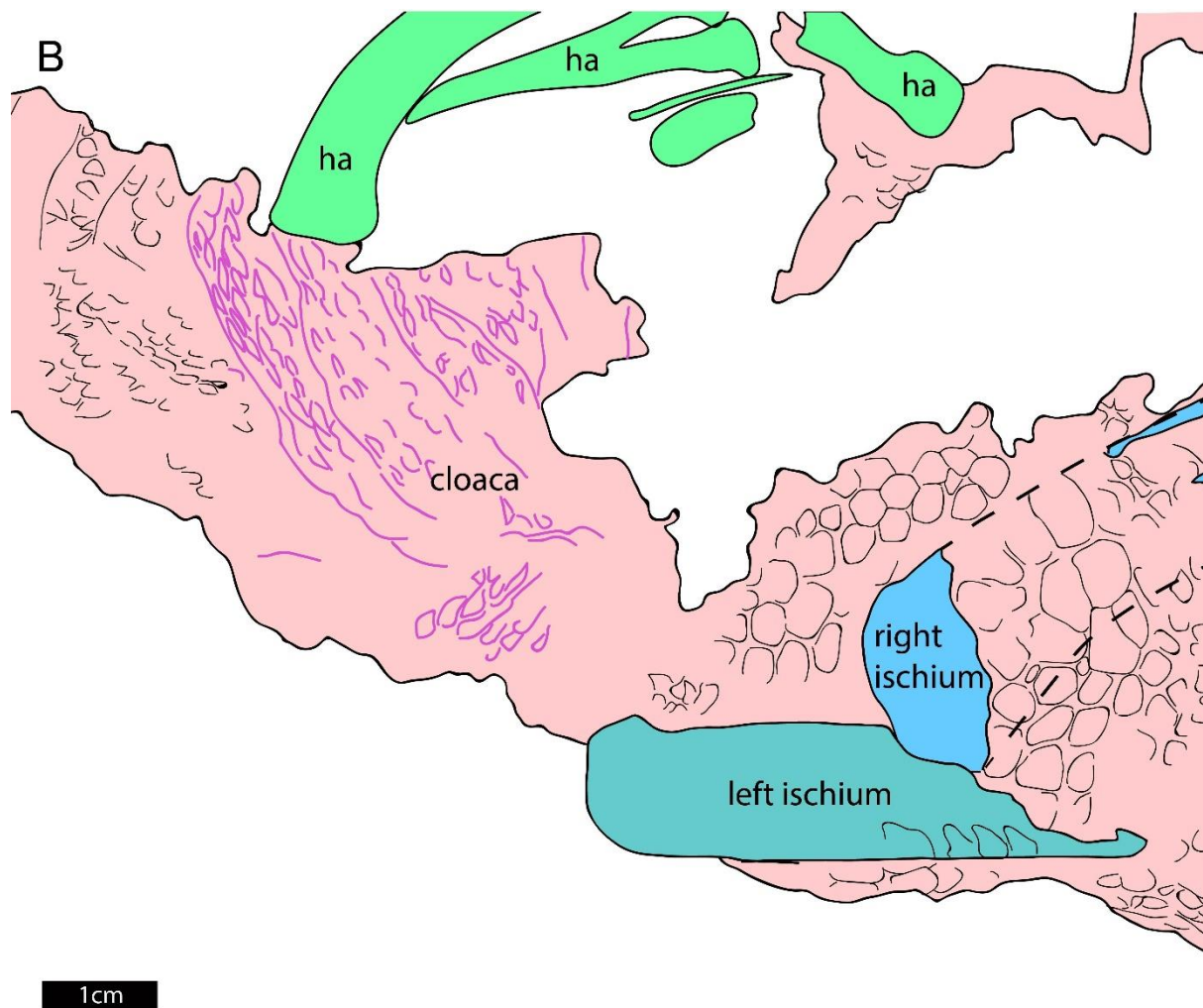
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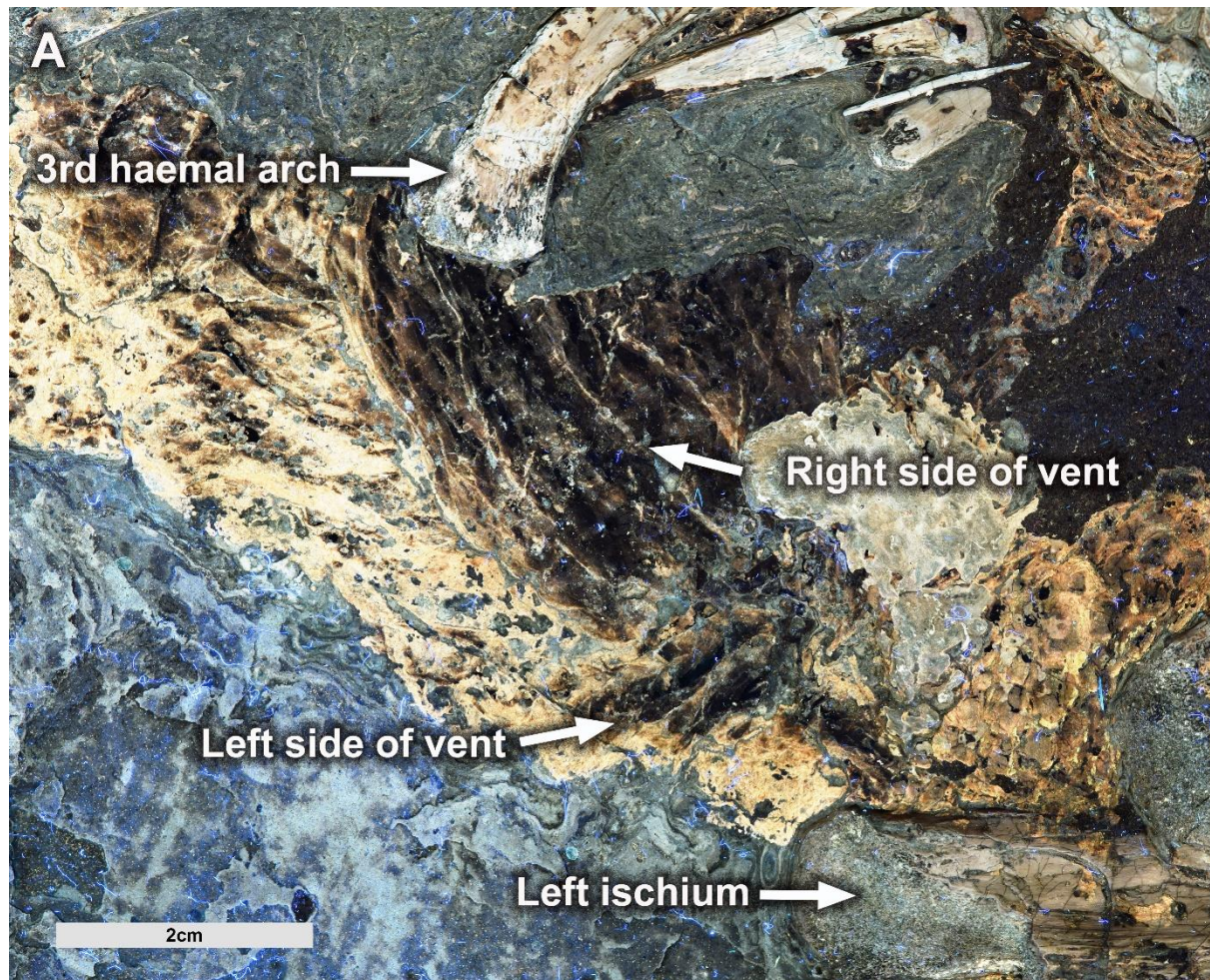
Figure 2. Close up of the cloaca of *Psittacosaurus* sp. SMF R 4970. A. The right side of

the longitudinally presented vent is better exposed. The outer morphology of the cloaca is

darkly pigmented compared to adjacent areas of the ventrum. Scale bar is 2cm. **B.**

Interpretative drawing of the cloacal region showing the integument in red shading and the

details of the cloaca in pink line colour. Haemal arches (ha) marked in lime green and left

and right ischia coloured in dark green and blue, respectively. Scale bar is 1 cm.

The darkly-pigmented tissue surrounding the vent is wrinkled, the creases of which are

parallel and radiate from the vent in a posterolateral direction (~3 cm long) towards the

ventral tip of the third haemal arch (Fig. 2). Unlike the other haemal arches, the third haemal

arch, which articulates with the posteroventral corner of the 7th caudal vertebra, is subvertical

94   and strongly bowed (anteriorly concave) (Fig. 1). In contrast, the remaining haemal arches

95   are all straight and strongly posteroventrally oriented (Fig. 1). The first and second haemal

96   arches are also peculiar in being roughly half the length of the third arch, although the first

97   arch appears to be broken into two adjacent lengths (Fig. 1). The scales covering the darkly

98   pigmented tissue surrounding the vent are also distinct from those on the abdomen and tail:

99   scales surrounding the vent are relatively large, roughly lenticular ($\bar{x}$ length = 3.4 mm), the

100   long axes of which are oriented parallel to the surrounding wrinkles (Figs. 1, 2). Further

101   along the tail, the ventral integument consists of distinct vertical bands (arranged transversely

102   in life) of typically rounded-quadrangular scales, which range from 1.7 to 3.3 mm in height

103   (Fig. 1). The same transverse banding of quadrangular scales also occurs on the abdomen,

104   anterior to the ischiadic symphysis (Fig. 1).

105

Discussion

106

107   *Cloaca anatomy and biological implications*

108   The *Psittacosaurus* specimen SMF R 4970 is unique among non-avialan dinosaurs in

109   preserving the outer morphology of the cloaca, including the vent. SMF R 4970 demonstrates

110   unequivocally that the genitals were housed internally in *Psittacosaurus* and likely in all

111   other non-avialan dinosaurs, as in all known animals with a cloaca. For comparison with the

112   cloaca of SMF R 4970, a representative sample of snakes, lizards, birds, and crocodylians

113   were observed in the collections of the University of New England's Natural History

114   Museum. The internal anatomy of the cloaca in lepidosaurians, crocodylians, and birds

115   differs substantially and is accompanied by modifications to the external appearance of the

116   vent. The vent in living sauropsids can be divided into three main types [17]: transversely

117   opening (snakes and lizards), longitudinally opening (crocodylians), or round/square (birds).

118   The integumentary covering across these three types differs accordingly. In snakes, the

119   transverse vent is covered by one or two cloacal scales that are modifications of the broad

120   ventral scales present elsewhere on the underbelly. The condition in lizards is highly variable

121   but the vent is always transverse and accompanied by a variable number of cloacal scales that

122   may or may not differ from the surrounding scales. Among birds, the area immediately

123   surrounding the cloaca is naked, bearing neither scales nor feathers. In crocodylians, the

124   longitudinal vent is surrounded by elliptical-to-polygonal scales that radiate and increase in

125   size from the vent itself. This rosette arrangement of cloacal scales is distinct from the

126   transverse rows of comparatively large quadrangular scales that extend along the ventral

127   surfaces of the abdomen and tail. Thus, the gross morphology of the vent in *Psittacosaurus*,

128   which combines a longitudinally opening vent with a rosette pattern of cloacal scales and

129   transverse rows of quadrangular ventral scales, most closely matches that of crocodylians.

130   The position of the vent is also distinctive in *Psittacosaurus.* Romer [1] remarked that

131   the cloaca opens immediately posterior to the distal ischia in all reptiles; however, it may be

132   supported by an ossified hypoischium that projects posteriorly from the ischial symphysis in

133   some lizards (see [18] and references therein). In many lizards, the vent is typically

134   positioned just posterior to the joint between the first and second caudal vertebrae and is

135   never further than the third caudal vertebra [18]. Birds on the other hand have highly

136   modified tails that terminate in a pygostyle (e.g. [19]) and therefore do not provide a suitable

137   analogue for comparisons. Matching Romer's [1] prediction, the vent, in *Psittacosaurus*,

138   opens just posterior to the distal ischia and in line with the joint between the fifth and sixth

139   caudal vertebrae.

140   The crocodilian-like vent of *Psittacosaurus* implies that, unlike lizards and later-

141   diverging birds, *Psittacosaurus* probably had a muscular, unpaired, and ventrally-positioned

142   copulatory organ (e.g. [20]) and a ureter that was decoupled from the copulatory organ [9].

143   Some birds possess a single copulatory organ, but the majority of species lack a phallus

144   entirely [21]. Like crocodylians, birds still use internal fertilisation, which is the presumed

145   method in *Psittacosaurus*. Crocodylians and some early-diverging crown birds (e.g., *Rhea*,

146   tinamous) have a ureter that opens into the coprodeum, whereas in later-diverging crown

147   birds, the ureter empties into the urodeum [9]. The presumably paired oviducts in

148   *Psittacosaurus* [22] would have opened into the urodeum as well.

149   Despite the remarkable preservation of SMF R 4970, it was not possible to determine

150   its sex. Although histological examination was not available, SMF R 4970 was not gravid,

151   and therefore unlikely to have been producing medullary bone [23]. There is no evidence of a

152   recognisable sexually dimorphic cloacal structure in SMF R 4970. A cloacal protuberance

153   temporarily develops in some birds during the breeding period, but its utility as a dimorphic

154   structure is undermined by its presence in both males and females in some species [24]. In

155   crocodylians, sex determination is entirely dependent on the inspection of the genitalia and

156   has no relationship to the external morphology of the cloaca/vent [25, 26]. Internally, a

157   reduced first (and second) haemal arch—as in SMF R 4970—has been suggested to identify

158   female dinosaurs based on the alleged condition in modern crocodylians [1, 27]. However,

159   this hypothesis has no statistical support [28]. The enlarged third haemal arch was likely the

160   origin for muscles responsible for closing the cloaca (m. transversus medianus [17]). Anterior

161   haemal arches are commonly abbreviated in dinosaurs and seem to be an ancestral feature

162   based on rare early dinosaur fossils preserving this portion of the tail (e.g. *Chilesaurus*,

163   *Eoraptor*, and *Tawa*). Their presence in modern crocodylians suggests they might even be

164   ancestral to archosaurs pending further fossil tail discoveries. Thus, the only known record of

165   a non-avialan dinosaur cloaca has a crocodylian-style and does not show external sexually

166   dimorphic structures that can determine the sex of SMF R 4970.

167

168   **Methods**

169   SMF R 4970 was photographed using Laser-Stimulated Fluorescence (LSF) performed using

170   an updated version of the methodology proposed by Kaye *et al*. [29] and refined in Wang *et*

171   *al.* [30]. Only a brief description of the method is provided here. A 405 nm violet near-UV

172   laser diode was used to fluoresce the specimen following standard laser safety protocol. Long

173   exposure images were taken with a Nikon D810 DSLR camera fitted with a 425 nm long-

174   pass blocking filter and controlled from a laptop using *digiCamControl*. Image post-

175   processing (equalization, saturation and colour balance) was performed uniformly across the

176   entire field of view in *Photoshop CS6*. As soft tissue details in SMF R 4970 are best visible

177   under LSF, the observations made using this technique and the resulting digital images

178   formed the basis for our descriptions. All measurements were taken using digital images

179   uploaded and calibrated in *ImageJ v1.52q* or *Photoshop CS6*. Comparative anatomy involved

180   a representative sample of snakes, lizards, birds and crocodylians, which were observed in

181   the collections of the University of New England's Natural History Museum (Armidale,

182   Australia). Computed tomography (CT) scans of a subadult male *Crocodylus porosus*

183   scanned and acquired by Klinkhamer *et al.* [31] were visualised using *Dragonfly 2020*.1

184   (www.theobjects.com).

185

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