

1 **Shell Morphology, Radula and Genital Structures of New Invasive Giant African Land**
2 **Snail Species, *Achatina fulica* Bowdich, 1822, *Achatina albopicta* E.A. Smith (1878) and**
3 ***Achatina reticulata* Pfeiffer 1845 (Gastropoda:Achatinidae) in Southwest Nigeria**

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19 **Abstract**

20 The aim of this study was to determine the differences in the shell, radula and genital
21 structures of 3 new invasive species, *Achatina fulica* Bowdich, 1822, *Achatina albopicta* E.A.
22 Smith (1878) and *Achatina reticulata* Pfeiffer, 1845 collected from southwestern Nigeria and to
23 determine features that would be of importance in the identification of these invasive species in
24 Nigeria. This is the first report of *Achatina albopicta* and *A. reticulata* in Nigeria, but *Achatina*
25 *fulica* have since been reported in Nigeria and other African countries outside coastal East
26 Africa. No study has described the external or internal morphology of any of the invasive species
27 in Nigeria. Five to ten live specimens of each species, with complete shell characters, of each
28 species were used for this study. Vernier caliper was used to obtain all shell measurements, with
29 the shell held vertically and the aperture facing the observer. The genital structures were
30 dissected out and fixed in 70% alcohol for 10-15 minutes and examined. The buccal mass was
31 dissected out and digested in 7.5% sodium hydroxide for 24 hrs to free the radula from snail
32 tissues and then examined under the compound microscope.

33 The shells of the 3 new species were dextral, conical with pointed spire and narrow apex.
34 The whorls were separated by deep sutures. The parietal walls and the columella of the three
35 species were white but columella of *A. reticulata* had a characteristic thick deposit of white
36 porcelain-like material. There were dark brown markings on the whorls of the three species on
37 dirty brown background for *A. fulica* and *A. reticulata* and dirty yellowish background for *A.*
38 *albopicta*. The shell of *A. albopicta* was slightly glossy on the body whorl. The whorls of *A.*
39 *albopicta* were much more convex than the whorls of *A. fulica* and *A. reticulata*. The columella
40 of *A. albopicta* was truncate above the base of the peristome, moderately concave and slightly

41 curved up at the base, while the columella of *A. fulica* was truncate sharply at the base of the
42 peristome and straight and the columella of *A. reticulata* was slantly truncate at the base of the
43 peristome and straight. The genitalia of the three species were very identical but differed slightly
44 in the emergence of the basal vas deferens from the penis. The penes were slender and
45 completely enclosed by the penial sheaths. The length of the penis varied from 10 to 12 mm. The
46 vas deferens, free oviduct and the spermatheca duct were very long. The radula could be
47 differentiated by the structure of central teeth and the first lateral tooth. The study showed that
48 the shell morphology, radula and genital structures can be of importance in the identification of
49 members of the family Achatinidae in Nigeria.

50 Key words: *Achatina fulica*, *Achatina albopicta*, *Achatina reticulata*, invasive species, shell
51 morphology, radula, genital structures.

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61 **Introduction**

62 Species of Achatinidae are endemic in African countries south of the Sahara. They are
63 characterized by large to medium sized, broadly ovate shells, with regular conical spires. With
64 increase in mobility of humans and globalization of travel and trade, several achatinids, have
65 been accidentally or purposefully transported to areas outside their native range, where they
66 cause significant economic and ecological impacts [1]. *Achatina fulica* Bowdich, 1822 and its
67 subspecies are native to the coastal East Africa, particularly Kenya and Tanzania but have now
68 been introduced to many other African countries like Cote d'Ivoire, Togo, Ghana, Nigeria [2-6],
69 Benin Republic where *A. fulica* has already overtaken the west African land giant snails in
70 population density [7], and many countries in tropical and subtropical regions [1].

71 The Giant African land snails *Achatina fulica* Bowdich, 1822 is considered to be among the
72 world's 100 worst invasive species and also ranked among the worst snail pests of tropical and
73 sub-tropical regions, causing significant damages to farms, commercial plantations and domestic
74 gardens [8]. It is an intermediate host of the rat lungworm, *Angiostrongylus cantonensis*, which
75 causes eosinophilic meningoencephalitis in humans and *Angiostrongylus costaricensis*, the
76 etiological agent of abdominal angiostrongylosis [9, 10]. *A. fulica* can consume several species of
77 native plants, agricultural and horticultural crops, modify habitats and outcompete native species
78 [11]. In Nigeria the West African species of *Archachatina* and *Achatina* (subgenus *Achatina*) are
79 usually favoured and they grow to reasonable sizes than any other known land snails. The genus
80 *Achatina* had been represented by *Achatina achatina* (Linne) and its subspecies, but in recent
81 times *Achatina fulica* (subgenus *Lissachatina*) has been reported in some parts of the

82 country [5,6]. A recent study of the live specimens of *Achatina* spp collected from Itori, in
83 Ogun state and the University of Ibadan, Ibadan, Nigeria in Oyo state, showed that the samples
84 collected were represented by three separate species (*Achatina fulica*, *Achatina albopicta* and *A.*
85 *reticulata*),following the original descriptions of Bequaert [12]. Shell morphology alone could
86 not reveal clearly the differences between these species, hence a combination of shell, radula and
87 genital structures known to be important in the classification molluscs [13] were investigated for
88 their significance in the identification of the invasive snail species. This study was undertaken to
89 reveal the differences in the shell, radula and genital structures of the three invasive species and
90 provide notes on characters that can be important for their identification in areas where they have
91 been recently introduced and to contribute to the available information on the terrestrial
92 molluscan species richness in Nigeria that is still poorly investigated.

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102 **Materials and Methods.**

103 **Snail samples**

104 The samples of *Achatina fulica* used for this study were collected from Itori, headquarter of
105 Ewekoro Local Government Area in Ogun State, Nigeria, located at 6°93'23"N and 3°22'47"E.
106 The average monthly temperature ranges from 23°C to 32.2°C and it is a tropical rain forest area
107 undergoing transition to guinea savannah due to farming and mining activities. The samples of
108 *Achatina albopicta* and *A. reticulata* were collected from the residential areas of University of
109 Ibadan, Ibadan, Oyo state, Nigeria , located at 7° 23'28.19" and 3° 54'59.99" E "Fig.1". Five to
110 ten live snails were used for the study. Snails were identified using Bequaert [12].

111 **Snailery**

112 Snail samples collected from the field were maintained in the snailery in the Department of
113 Zoology, University of Ibadan, Ibadan, Nigeria, before examination. Snails were kept in
114 rectangular glass containers (93 x 62 x 58 cm), with metal mesh covers, field half way with
115 moist humus soil. The snails were maintained under the natural regime of 12hr light and 12hr
116 darkness, fed *ad libitum* with *Carica papaya* leaves, *Tridax triangulare* leaves and unripe
117 pawpaw fruits before examination.

118

119 **Fig 1. Map of Oyo and Ogun States Showing the Collection Sites for the Invasive *Achatina***
120 **Species in Nigeria**

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122 **Shell morphometrics**

123 Vernier caliper was used to obtain the conchological character measurements [14]. All
124 measurements were taken with the shell held vertically with the aperture facing the observer, and
125 only complete shells i.e. shells with no missing parts, were used for the study. The shell height
126 (SH) was the longest vertical axis of the shell, measured from the tip of the spire to the basal
127 edge of the outer lip. The shell width (SW) was the largest diameter, measured at right angle to
128 the vertical axis, from the left margin of the body whorl to the outer edge of outer lip. The
129 aperture height (AH) was the longest distance from the insertion of the outer lip on the parietal
130 wall to the basal edge of the outer lip, while the aperture width (AW) was the greatest distance
131 from the inner edge of the columella to the edge of the outer lip. The spire height (SpH) was
132 measured from the tip of the apex to the suture separating the spiral whorls from the body whorl,
133 and the body whorl height was the measured from the suture separating the spire from the body
134 whorl to the base of the peristome (BwH). All measurements were in millimeters.

135 **Shell parameter indices**

136 From the values obtained for each linear measurement, the following indices were determined,
137 following the methodology of Medeiros et al.[15]: the shell height/shell width (SH/SW); body
138 whorl height/shell width (BwH/SW); shell aperture height/shell aperture width (AH/AW); and
139 spire height/ body whorl height (SpH/BwH).

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142 **Number of whorls**

143 The number of whorls corresponds to the number of complete turns of the shell beginning from
144 the embryonal whorls at the tip of the spire.

145 **Dissection of genital structures**

146 Each specimen was placed in a jar filled with boiling water for 10 – 15 minutes to loosen the
147 columellar muscle and the soft body was extracted from the shell with hooked metal. The shell
148 was preserved for shell morphometrics. The genital structure was dissected out of the soft body
149 and fixed in 70% alcohol for 10 minutes and examined. The genital structure was then preserved
150 in 10% formalin.

151 **Radula preparation**

152 The buccal mass was dissected out of the head region of the soft body and macerated in 7.5%
153 sodium hydroxide for 24 hours at room temperature. The freed radula was washed in water and
154 any leftover tissues were removed under the dissecting microscope. The radula was mounted in
155 glycerine and view under the compound microscope. After observation the radula was preserved
156 in 70% alcohol. Photographs of radula were taken with UCMOS series microscope camera
157 MU500 with Toupview 3.2 image software.

158 **Statistical analysis**

159 To detect variations in shell morphology between species, the linear measurements and ratios
160 were subjected to logarithmic transformation (\log_{10}) before using Analysis of Variance
161 (ANOVA, $p \leq 0.05$).

162 **Results and Discussion**

163 The three new species in this study, *Achatina fulica*, *A. albopicta*, and *A. reticulata* were
164 collected from residential areas in Ibadan, an urban community and from farmlands in Itori, a
165 semi urban community in southwest Nigeria. The invasive species are known to thrive in the
166 presence of man, especially in urban sites and farms or disturbed areas [8], and they have
167 superior competitive abilities over endemic species, whose distributions are often affected, due to
168 the high fecundity and reproductive rates of the invasive species [11]. This invasion will
169 significantly impact the species richness of land snails in Nigeria. It is therefore important that
170 the extent of this invasion in Nigeria is monitored and properly managed before they assume pest
171 status or dominance over the West Africa species of achatinidae that have served as cheap
172 sources of protein for the rural populace and have not been incriminated in the transmission of
173 any known parasitic infections of public health importance. The invasive *A. fulica* has achieved
174 dominance in the achatinid communities in Ivory Coast, Ghana and central Benin Republic [1,
175 7]. While it is not precisely known how the invasive species got to Nigeria; it is most likely that
176 they were introduced for economic reasons and subsequent uncontrolled uses may increase their
177 ranges in future as they are moved around for sale, food or farming by humans. What should
178 also be of concern, apart from the effect on ecology and biodiversity, is that the invasive species
179 can act as intermediate hosts of Metastrongylidae nematodes, particularly now that
180 *Angiostrongylus cantonensis* Cheg, 1935, which causes eosinophilic meningitis is spreading
181 rapidly to many parts of the world [10].

182 The shell morphology of the three invasive species (*Achatina fulica*, *A. albopicta* and *A.*
183 *reticulata*) in this study conformed to the original descriptions of the three species from East

184 Africa [12]. The shells were dextral, conical with pointed spires and narrow apex. They had a
185 minimum of 8 whorls and the whorls were separated by deep sutures. The parietal walls and
186 columella of the three species were whitish. There were dark brown markings on the whorls of
187 the three species, but on dirty brown background for *A. fulica* “Fig 2” and *A. reticulata* “Fig 3”,
188 and dark brown or yellowish background for *A. albopicta* “Fig 4”, which was also slightly glossy
189 on the body wall. The major differences in shell features are shown in “Table 1”.

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191 **Fig 2. Apertural and abaperural views of shell of *Achatina fulica* collected**

192 **From Itori, Ogun State, Nigeria. Scale bar = 1.0cm**

193 **Fig 3. Apertural and abaperural views of shell of *Achatina reticulata* collected**

194 **from Ibadan. Scale bar = 1.0 cm**

195 **Fig 4. Apertural and abaperural views of shell of *Achatina reticulata* collected**

196 **from Ibadan. Scale bar = 1.0 cm**

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198 The three invasive species exhibited considerable morphometric similarities “Table 2”. They
199 were high-spired species (shell height > shell width), and the shell spired index (SH/SW) ranged
200 from 1.8-2.0. The shell of *A. fulica* was most slender (SH/SW=2.0±0.04) followed by the shell of
201 the shell of *A. reticulata* (SH/SW=2.0±0.1) and *A. albopicta* (SH/SW=1.8±0.1). However, the
202 mean values of the shell parameters were significantly ($p < 0.05$) larger for *A. reticulata* than *A.*
203 *fulica* and *A. albopicta* “Table 2”. The shells of *Achatina reticulata* had a mean height of

204 134.0.3± 17.4 mm and was larger than the shell of *A. fulica* (90.0 ± 2.7 mm) and *A. albopicta*
 205 (104.1± 7.6 mm) “Table 2”. The aperture index (AP/AW) ranged from 1.9-2.3 among the three
 206 species. The shell shapes fit into the patterns described for invasive achatinidae [16]; this study
 207 showed that the three species had elongated spire with narrow body whorl and narrow aperture.
 208 *Achatina reticulata* was the largest of the three species in this study with conspicuous
 209 longitudinal sculptures on the body whorl. The high shell-spired indices of the three species may
 210 account for the ease with which they burrow or burry in the soil.

211 **Table 1. Showing differences in shell characters of the invasive Achatina species in**
 212 **southwestern Nigeria.**

Shell characters	<i>Achatina</i> species		
	<i>Achatina fulica</i>	<i>Achatina albopicta</i>	<i>Achatina reticulata</i>
Spire	The spire occupied 30% of the total height of the shell, with longitudinal brown stripe or patches. The whorls were slightly convex.	The spire occupied 32% of the total shell height, with light brown irregular patches or stripes. The whorls were strongly convex.	The spire occupied 29 % of the total shell height, with dirty white background and zigzag/ longitudinal brown patches or stripes. . The whorls were strongly convex.
Body whorl	Slightly glossy and terminates almost at the end of the columella.	Slightly glossy, and extends below the truncated end of the columella.	Very rough with longitudinal sculptures visible to the naked eyes.
Columella	White, nearly straight and sharply truncated at the base of the aperture.	White, concave, bent upwards and sharply truncated above the base of the aperture.	White porcelain-like deposit, straight and strongly truncated at a slant above the base of the aperture.
Parietal wall	Dark brown background with faint white	White with faint dark brown background	Thick white porcelain-like deposit
Inside of shell	White with bluish tinge on a dark brown background	White with bluish tinge	Very white with bluish tinge

Outer lip of peristome	Blackish brown and thin	Light brown and thin	Thick and white with broken brown patches
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214 **Table 2. Morphometric characterization of invasive Achatinidae collected from**
 215 **southwestern Nigeria**

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Character/Index	<i>Achatina fulica</i>	<i>Achatina albopicta</i>	<i>Achatina reticulata</i>	
	X±SD(Range) mm	X±SD (Range) mm	X±SD (Range) mm	P value
No. of whorls	8.3±0.1(8.25-8.5)	8.3±0.1(8.25-8.5)	8.4±1.4 (8.25-8.5)	F=0.303;p=0.741 ^a
Shell height(SH)	90.0±2.7(87-93)	104.1±7.6(98-120)	134.0±17.4 (103-145)	F= 200.1;p=0.0001
Shell width(SW)	44.4±0.8(43-45)	56.4±4.4(52-65)	70.6±3.0 (69-75)	F=374.4;p=0.0000
Spire height(SpH)	27.4±2.5(25-31)	30.5±1.3(29-32)	41.0±1.0(40-42)	F=54.0;p=0.0000
Body whorl height(BwH)	57.3±3.9(52-64)	77.0±5.8(70-85)	88.8±2.3(88-92)	F=0.0949;p=0.9098 ^a
Aperture height(AH)	44.4±0.7(43-45)	53.1±3.2(50-58)	56.0±1.2(55-58)	F=104.8;p=0.0000
Aperture width(AW)	22.0±1.2(20-24)	23.0±2.4(21-28)	30.6±1.5(29-33)	F=64.0;p=0.0000
SH/SW	2.0±0.1(2.0-2.1)	1.8±0.1(1.8-1.9)	2.0±0.1(1.9-2.0)	F=27.1;p=0.0000
AH/AW	2.0±0.1(1.9-2.1)	2.3±0.1(2.1-2.5)	1.9±0.1(1.8-2.0)	F=28.9;p=0.0000
BwH/SH	1.3±0.1(1.2-1.5)	2.3±0.1(2.1-2.5)	1.9±0.1(1.8-2.0)	F=1.157;p=0.335 ^a
SpH/BwH	0.5±0.0(0.4-0.5)	0.4±0.0(0.4-0.4)	0.5±0.0(0.5-0.5)	F=41.1;p=0.0000

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218 ^aNon-significant difference between mean values

219 The genitalia of three species were very identical, the basal genital structures were similar;
220 the penes were slender and completely enclosed by the penial sheath “Fig 5”. The length of the
221 penes varied from 10 mm to 12 mm in the three species “Table 3”. The vas deferens, free oviduct
222 and the spermatheca duct were very long. The basal uterus is slightly greenish and the apical
223 uterus is yellowish to pale cream. The hermaphroditic duct is highly convoluted and the basal
224 part is pale cream in colour while the apical part is black in colour. The major differences in the
225 basal genital structures are shown in “Table 3”. The genital structures were significantly
226 different from the genital structures of the West African Achatinidae [17].

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228 **Fig 5. Diagrammatic illustrations of the basal portion of the genital system of *Achatina***
229 ***albopicta* (A), *Achatina reticulata* (B), *Achatina fulica* (C), showing the basal portion of**
230 **uterus and prostrate (a), spermatheca (b), spermatheca duct, basal portion of the vagina**
231 **(d), penis (e), basal portion of vas deferens (f) and the flagellum (g). Scale bar = 1.0 cm**

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234 **Table 3. Main features of the basal genital structures of *Achatina fulica*, *Achatina albopicta***
235 **and *Achatina reticulata* collected from southwestern Nigeria**

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Basal Genital structures	<i>Achatina fulica</i>	<i>Achatina albopicta</i>	<i>Achatina reticulata</i>
Penis	10-11mm long	10-11 mm long	11-12 mm long
Penis sheath	Completely encloses the penis; basal portion of penis is swollen	Completely encloses the penis, swollen at the extreme end joining the basal vagina	Completely encloses the penis
Basal vas deferens	Emerges from the sheath almost at the upper end of sheath	Emerges from the sheath at the upper ¼ of the sheath	Emerges from the sheath close to the middle of the sheath
Basal vagina	Swollen and corm-like	Swollen and corm-like	Swollen and corm-like

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245 The radulae of the three species followed the basic pattern for the pulmonata. There were a set of
 246 teeth on either side of the median tooth, the lateral teeth and another set of marginal teeth. The
 247 median teeth in the three species were very small compared to the lateral and marginal teeth and
 248 they were bicuspid. The shape of the median tooth differed in the three species “Table 4”. The
 249 lateral teeth of the three species were usually tricuspid, with a large centrally located cusp
 250 (mesocone) and two poorly developed accessory cusps (endocone and ectocone) on both sides of
 251 the central cusp. However, the first lateral tooth on the right side of the median tooth of *Achatina*
 252 *reticulata* “Fig 6” had well developed accessory cusps (endocone) next to the median tooth; the
 253 ectocone was not conspicuous. The radulae could be differentiated by the structures of their
 254 median teeth “Figs 6A, 7A, and 8A”. The accessory cusps, increased in number and levels of
 255 development from the first lateral tooth toward the margin, in the three species. The marginal
 256 teeth of the three species “Figs 6C, 7C and 8C” were characterized by larger number of cusps,
 257 with the mesocone and the accessory cusps (ectocone and endocone) further divided into smaller

258 cusps. This study appears to be the first to present a detail study of the radula structures of the
 259 three invasive species. The radulae differed in the shape of median teeth, the development of the
 260 accessory cusps, particularly the endocone on the first lateral teeth.

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263 **Table 4. Main features of the radulae of *Achatina fulica*, *Achatina albopicta* and *Achatina***
 264 ***reticulata* collected from southwestern Nigeria**

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Radula structures	<i>Achatina fulica</i>	<i>Achatina albopicta</i>	<i>Achatina reticulata</i>
Median tooth	Bicuspid with two unequal cusps, the larger cusp was pointed and taller than the smaller one with flat surface	Bicuspid and the two cusps are nearly of the same heights and sizes, with flat surfaces.	Bicuspid with two unequal cusps, the smaller cusp was pointed and taller than the larger cusp. The median tooth was almost obscured by the well-developed ectocone of the first right lateral tooth.
Lateral teeth	Tricuspid with well-developed mesocone	Tricuspid with well-developed serrated mesocone	Tricuspid with well-developed mesocone. The ectocone of the first right lateral tooth was well developed.
Marginal teeth	Numerous with the ectocone and endocone further divided into smaller cusps	Numerous with the ectocone further divided into smaller cusps	Numerous with the ectocone further divided into smaller cusps and the endocone greatly reduced.

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269 **Fig 6. Micrograph of *Achatina reticulata* radula showing the bicuspid median tooth and the**
270 **first right lateral teeth with well-developed endocone (A), tricuspid lateral teeth (B) and**
271 **the marginal teeth (C). Scale bar = 1 μ m.**

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273 **Fig 7. Micrograph of *Achatina albopicta* radula showing bicuspid median teeth (A),**
274 **tricuspid lateral teeth and a serrated mesocone and the marginal teeth (C).**

275 **Scale bar = 1 μ m**

276 **Fig 8. Micrograph of *Achatina fulica* radula showing bicuspid median teeth (A), tricuspid**
277 **lateral teeth (B) and the marginal teeth (C). Scale bar = 1 μ m**

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280 **Conclusions**

281 This study has shown that the invasive achatinids have become established in Nigeria and
282 apart from *A. fulica*, other new subspecies *A. albopicta* and *A. reticulata* have been introduced.
283 The reproductive structures, shell morphology and radula structures can be useful in the
284 taxonomy of land snails. The study also suggests that the invasive species generally referred to as
285 *A. fulica* in other areas, where they have been introduced, may be a mixture of subspecies of
286 *Achatina* (subgenus *Lissachatina*) which were probably introduced together. It is therefore,
287 likely that the other subspecies of the subgenus *Lissachatina* may be more widely spread outside
288 East Africa than is currently documented due to misidentification.

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291 **Acknowledgments**

292 We thank Chris Odeh and TundeDisu, both of the Department of Zoology, University of
293 Ibadan, Ibadan, Nigeria for their technical assistance. We are also grateful to Mr. Femi Balogun
294 of the Department of Archaeology and Anthropology, University of Ibadan, Ibadan, Nigeria for

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348 **Supporting information**

349 S1 Table. Shell morphometrics of three invasive Achatinidae in southwest Nigeria

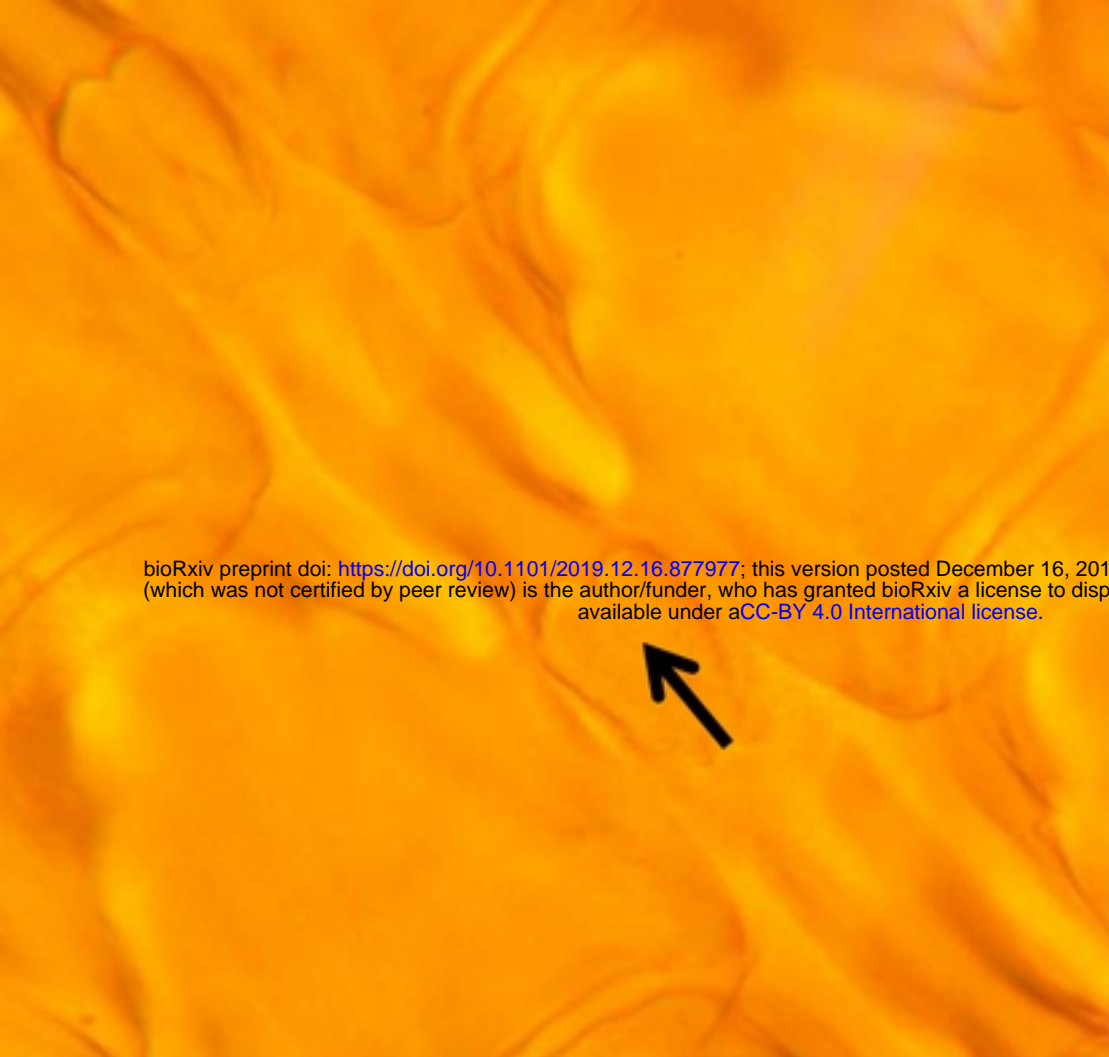
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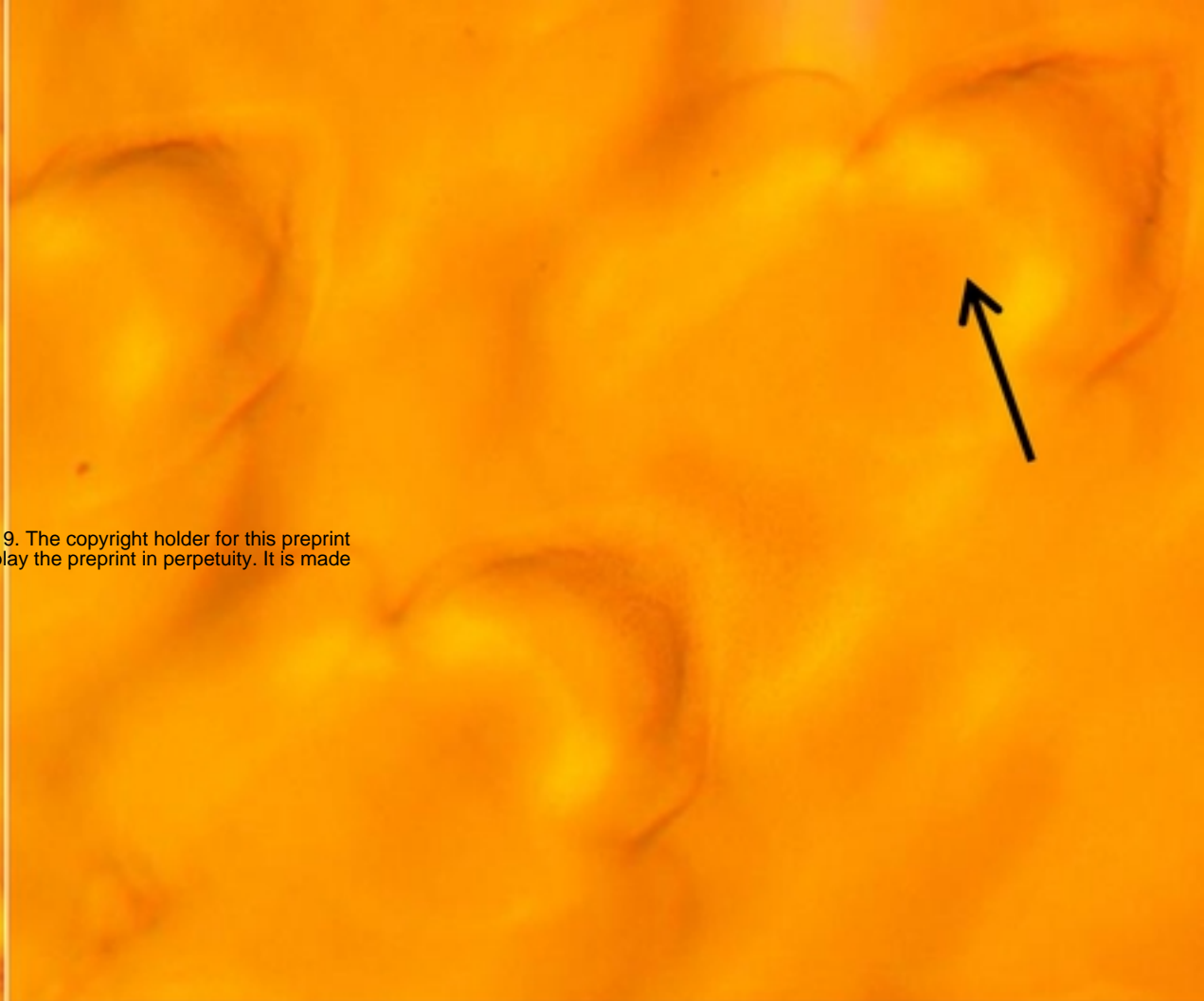


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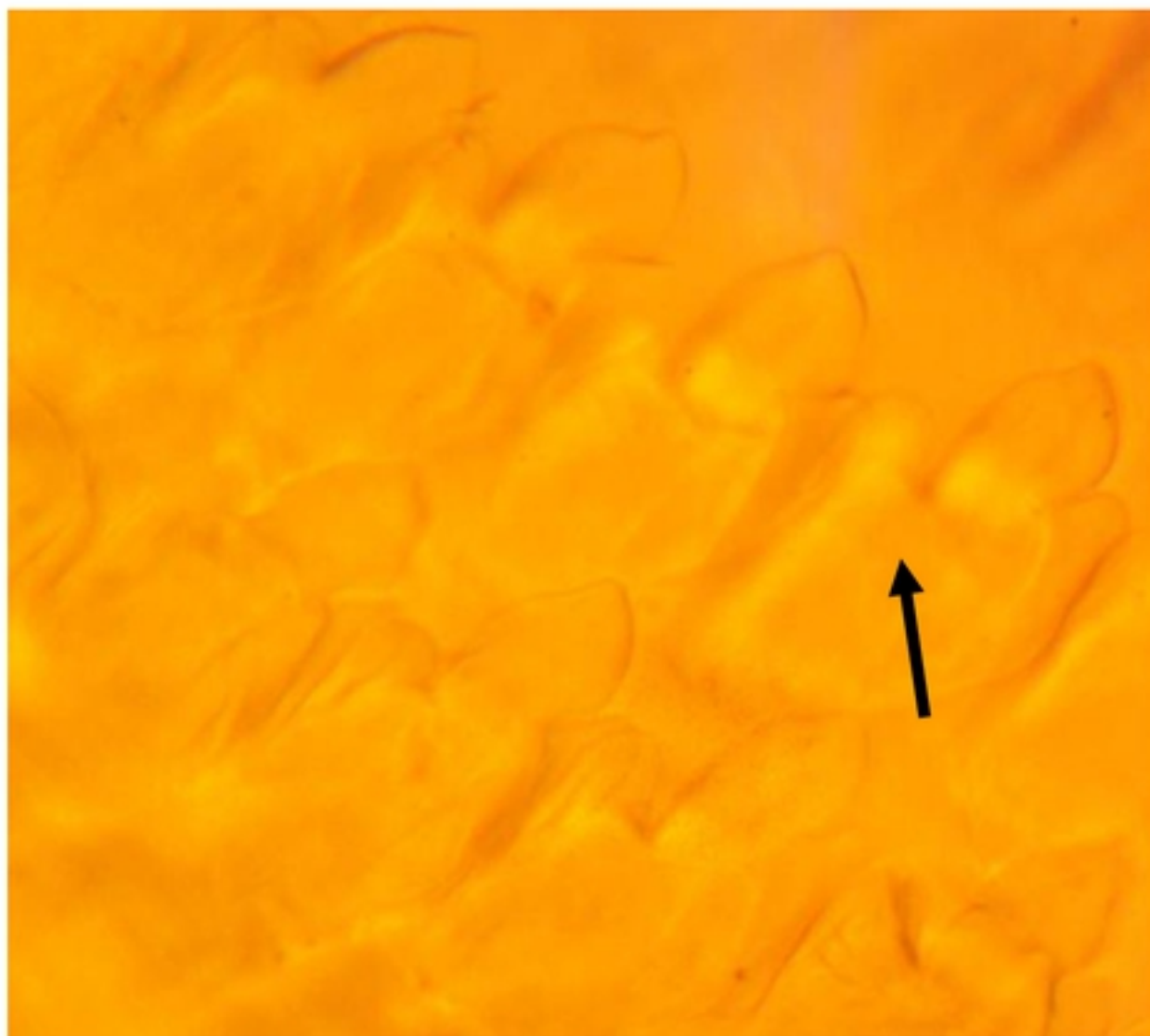
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A



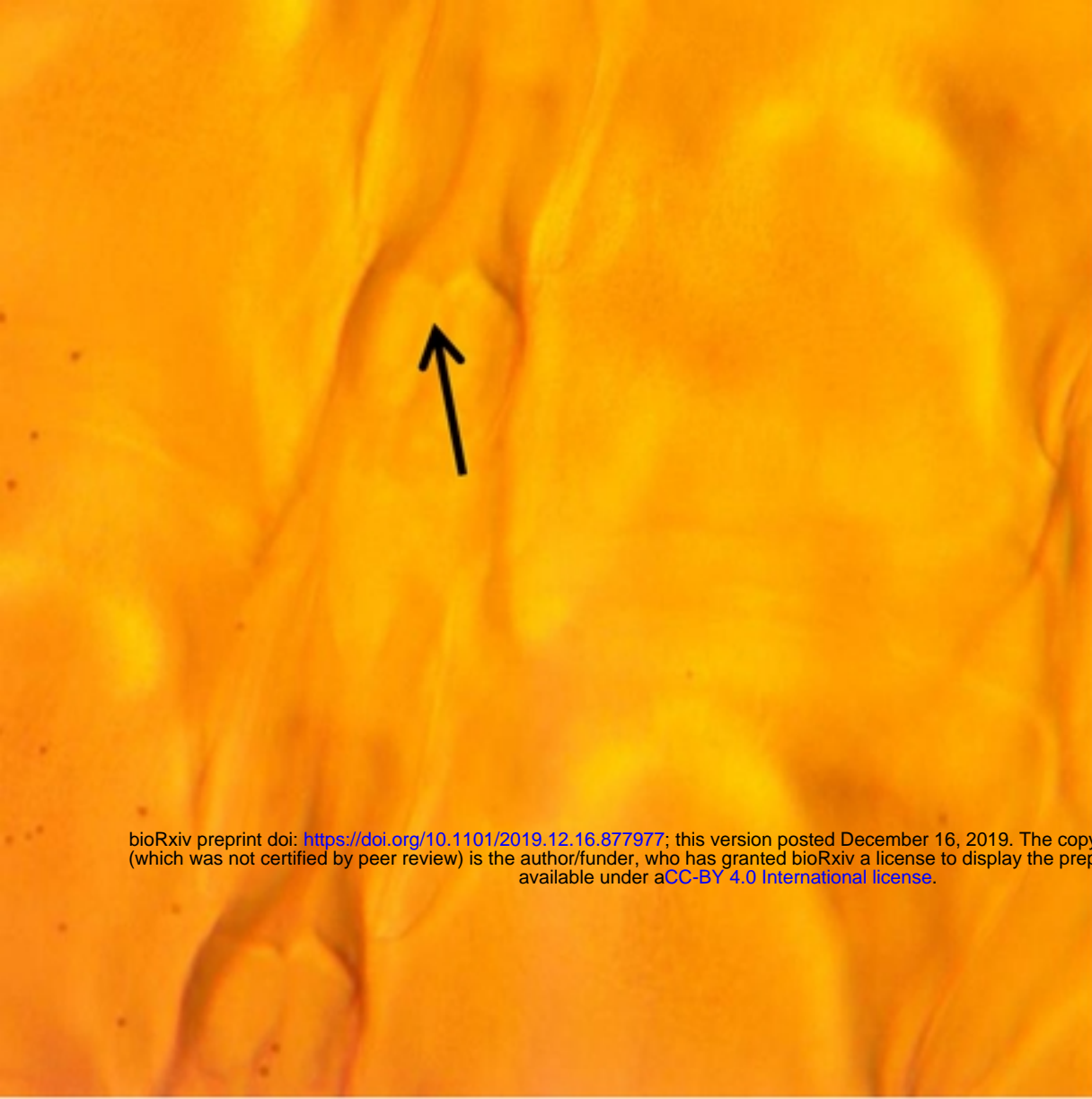
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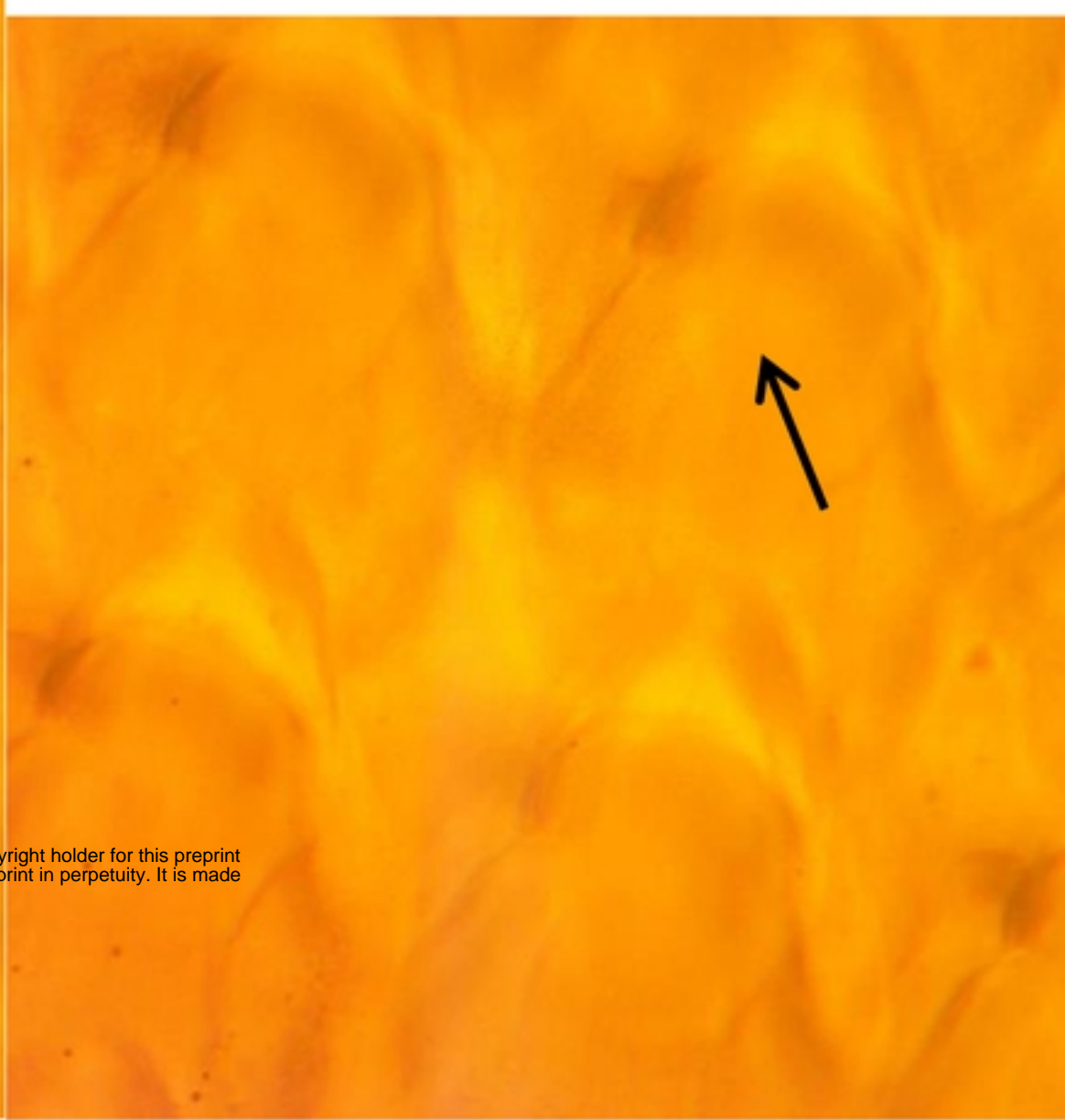
C

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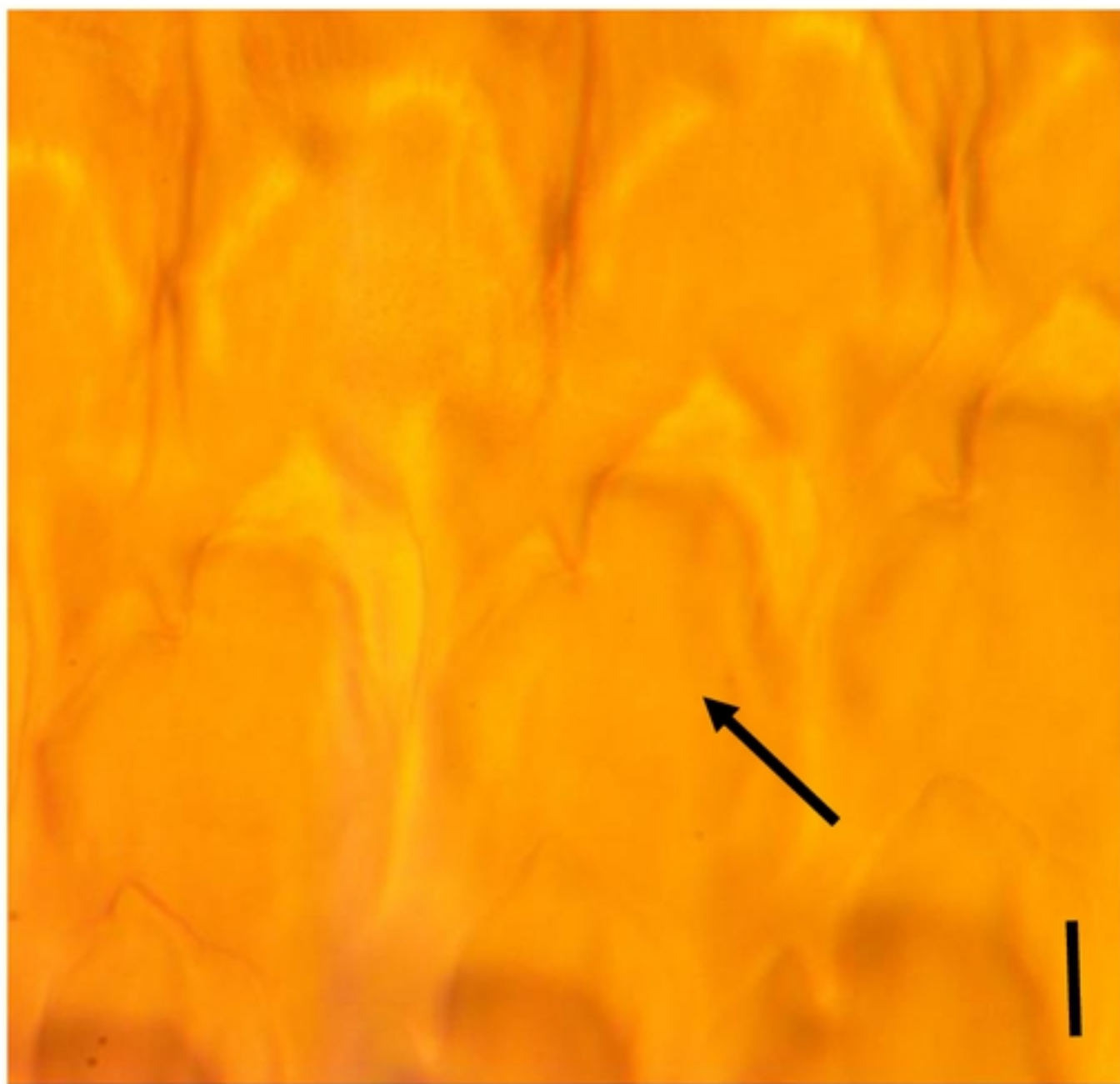
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A



B



C

Figure



Figure



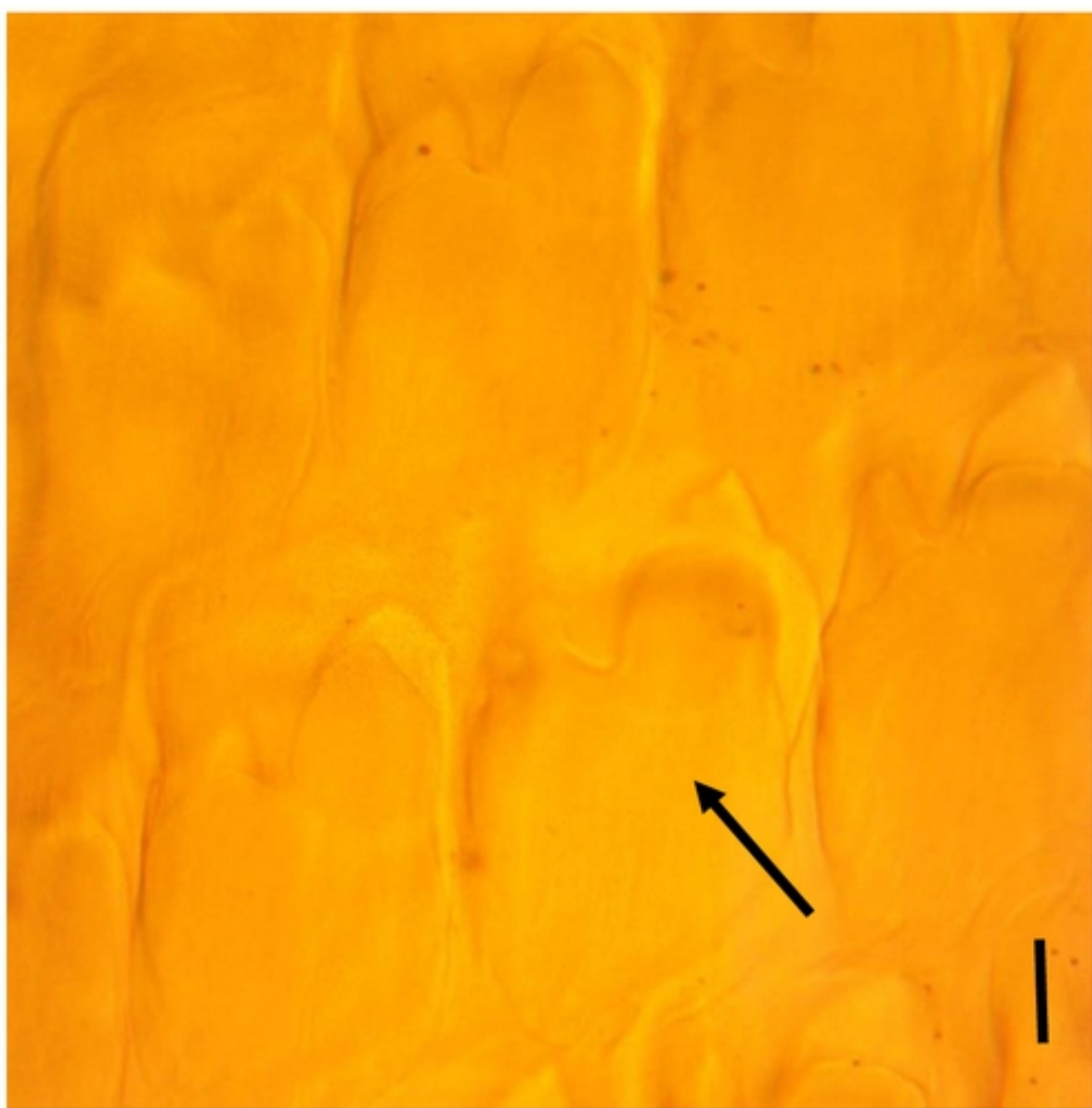
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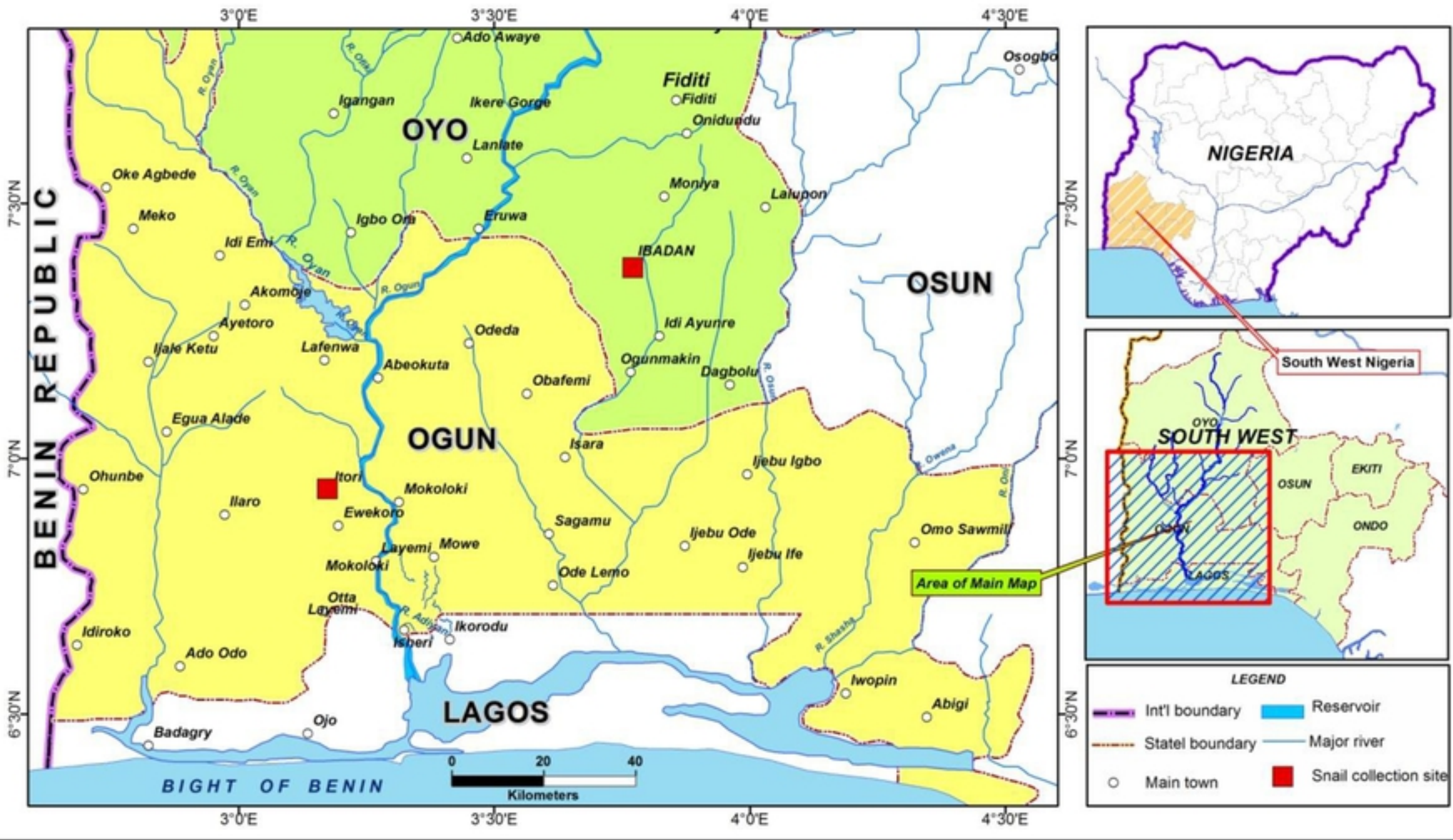
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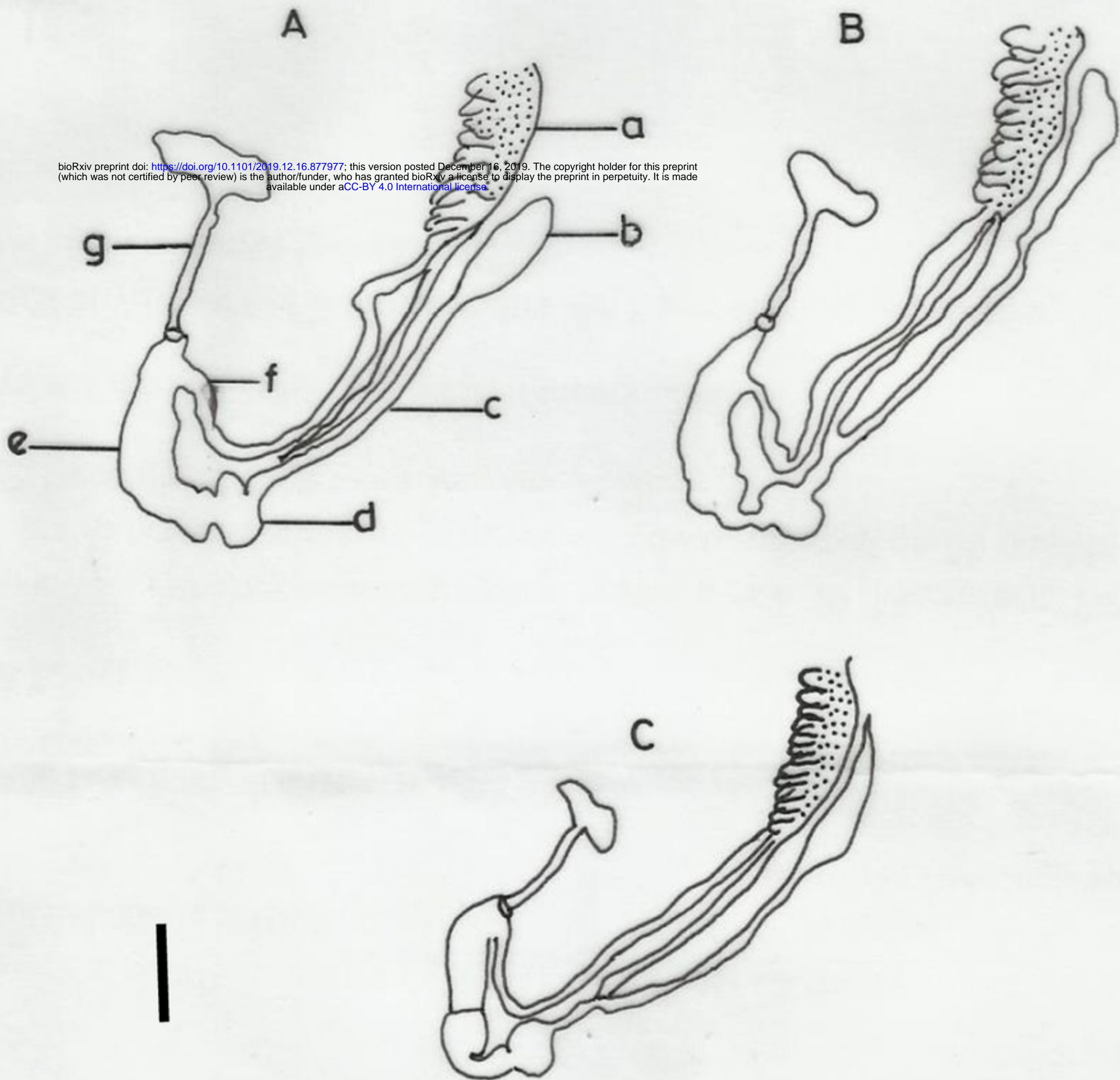
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