1　*Atypus karschi* Dönitz, 1887 (Araneae: Atypidae): an Asian purse-web spider established in

2　Pennsylvania, USA

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4　*Atypus karschi*: Asian spider established in the USA

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17

19 **Abstract**

20 The Mygalomorph spiders of the family Atypidae are among the most archaic spiders. The

21 genus *Atypus* Latreille, 1804 occurs in Eurasia and northern Africa, with a single enigmatic

22 species, *Atypus snetsingeri* Sarno, 1973, restricted to a small area in southeastern

23 Pennsylvania in Eastern USA. This study was undertaken to learn more about genetics of that

24 species, its habitat requirements and natural history. A close relationship to European species

25 could be assumed based on *A. snetsingeri'*s occurrence on the eastern coast of the USA,

26 however molecular markers (CO1 sequences) confirmed that *A. snetsingeri* is identical with

27 *Atypus karschi* Dönitz, 1887 native to East Asia; it is an introduced species. The specific

28 epithet *snetsingeri* is therefore relegated to a junior synonym of *A. karschi*. The karyotype of

29 *A. karschi* has 42 chromosomes in females and 41 in males (X0 sex chromosome system).

30 Chromosomes were metacentric except for one pair, which exhibited submetacentric

31 morphology. In Pennsylvania the above-ground webs are usually vertical and attached to the

32 base of bushes, trees, or walls, although some webs are oriented horizontally near the ground.

33 It was found in a variety of habitats from forests to suburban shrubbery, and over a wide

34 range of soil humidity and physical parameters. Prey include millipedes, snails, woodlice,

35 carabid beetles and earthworms. The number of juveniles in excavated female webs ranged

36 from 70 to 201. *Atypus karschi* is the first known case of an introduced purse-web spider. It is

37 rarely noticed but well-established within its range in southeastern Pennsylvania.

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Introduction

Mygalomorph spiders of the family Atypidae are among the earliest divergent groups of spiders (1). They dig a burrow and construct a 'purse-web', usually in the form of a closed tube, that occupies the burrow and extends above the ground horizontally or vertically for prey capture. The webs are well-camouflaged with soil particles and plant debris and potential prey are sensed when they walk on the surface of the tube. The spider impales the prey through the silk with its long fangs and injects paralytic venom. It then makes a slit in the tube large enough to drag the prey inside, repairs the tear with new silk, and feeds on the prey (2–5). Atypid spiders spend their entire lifetime within their burrow in the silken web, 8–10 years for some females, and enlarge the burrow as they grow (6–8). Males abandon their burrows when they reach maturity and wander in search of females, and then mate within the female's web. Egg-laying occurs within the maternal web and fully capable spiderlings emerge later. In contrast to most Mygalomorphs, atypid spiderlings utilize silk for aerial dispersal before establishing their first web (9,10). This ability may have allowed some species of atypids to colonize new areas, including those that were uninhabitable during the last glacial period (e.g., northern Europe, (11)).

There are currently three genera and 54 valid species of Atypidae (12). The genus *Atypus* Latreille, 1804 (34 species from Europe, Asia, North Africa and North America), spins an above-ground web that is tubular and typically lays horizontally and parallel to the soil surface. In *Sphodros* Walckenaer, 1835 (seven species from eastern North America) the above-ground web is tubular and usually attached vertically to trees and other vegetation. In the genus *Calommata* Lucas, 1837 (13 species from Africa and Asia) the above-ground web is a flat circular pouch set on the soil surface (13).

The center of diversity of the genus *Atypus*, based on the number of species, is in southeastern Asia and at least three species live in the western Palearctic. Despite the number and widespread distribution of *Atypus* species, they are secretive animals, and little is known about their habitat requirements, natural history, and genetic variation. In central Europe, particular *Atypus* species prefer sites with a microclimate regime resembling the climate of the glacial refuges from where they colonized the region (14). The species that live on open steppe habitats require soils rich in calcium that maintain a favorable air humidity in spider burrows. The species that do not require calcic soils occur only in habitats sheltered by woody

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vegetation, and their webs are hidden in detritus (15). As such, the European *Atypus* spiders are indicators of stable relic habitats and considered optimal flagship species in the conservation of disappearing relic xerothermic habitats (8).

Currently there are 16 Atypidae species with recorded DNA sequence data, eleven of which represent the genus *Atypus* (16). In contrast, only four species of Atypids, also in the genus *Atypus*, have been studied for their chromosomal constitution: *Atypus affinis* Eichwald, 1830; *Atypus karschi* Dönitz, 1887; *Atypus muralis* Bertkau, 1890; and *Atypus piceus,* Sulzer, 1776. The reported diploid number ranges from 14 to 44, and sex chromosome systems XY, X0, and X_1X_20 have been described (17–19). There are no data on other chromosome features, such as constitutive heterochromatin or nucleolus organizing regions (NORs). Those chromosome markers have been sporadically examined in Mygalomorphae (17,20).

This study looked at the genetics and habitat requirements of the lone species of *Atypus* found in North America, *Atypus snetsingeri* Sarno, 1973 (21). This spider appears to be restricted to a small geographic area near Philadelphia, Pennsylvania in eastern USA (22). It is morphologically similar to *Atypus karschi* of Asia (7,23,24) hypothesized that it was probably introduced to North America by human activity. To help resolve its relationship with other Atypids, the karyotype and genetic barcode (CO1) were developed for *Atypus snetsingeri* to compare with other *Atypus* species, along with observations on habitat associations and natural history.

Material and Methods

Study locations

In November 2013 we visited eight sites in Delaware County, Pennsylvania, that were known to have *Atypus snetsingeri* populations (Tessler, personal observations). The sites ranged from semi-urban areas near the Type locality to wooded county parks along riparian corridors where purse-webs were common. The primary site used for detailed web observations, specimen excavation and collection was a fallow field surrounded by forest at the Tyler Arboretum (Media, PA). That field was mowed annually to control invasive plants and facilitated access to the webs.

110 *Habitat and natural history*

111

112 At each site, we assessed the primary vegetation cover and soil type. The land orientation of

113 the web location was measured using a compass and the slope angles using an optical reading

114 clinometer to the nearest 0.5°. Soil penetration resistance was measured as described by Srba

115 & Heneberg (25), where higher values reflect mechanical impedance for burrowing.

116

117 The range of web sizes (tube diameter) was visually assessed in the field and prey were noted

118 by identifying remnants of invertebrates found attached to webs. The complete webs of 18

119 adult females were excavated on 5–9 November 2013. The length of the purse-webs were

120 measured, distinguishing the below-ground and above-ground sections by coloration and

121 attached soil. The size of the females was characterized by measuring the length of the

122 carapace along the midline. When spiderlings were present, their number was counted.

123 Voucher specimens from this study were deposited at the Crop Research Institute, Prague,

124 Czechia.

125

126 *Statistical analysis*

127

128 We used Pearson's correlation test to analyze the correlation between carapace size and tube

129 parameters (depth of the burrow, length of the capturing tube, and total length of the whole

130 web) and to analyze the correlation between individual tube parameters. We evaluated the

131 correlation between female size and number of offspring using the Spearman's correlation

132 test. The difference in body size between females with offspring and females without

133 offspring was analyzed using the Welch two sample *t*-test. We tested the two variances in the

134 subterranean and surface part of the tube by F-test. Normality was tested by Shapiro-Wilk

135 normality test. Data were analyzed in the statistical software R 3.6.2 (26). The means are

136 given with ± the standard error of the mean as a measure of sampling distribution.

137

138 *Karyotype analysis*

139

140 Chromosome preparations were obtained from gonads of one immature male (testes present)

141 and one mature female (ovary present). We followed the spreading technique described for

142 mygalomorphs by Král et al. (20) except for fixation time (10 and 20 min). The standard

143 preparations were stained by 5% Giemsa solution in Sörensen phosphate buffer for 25 min.

5

144   The evaluation of the karyotype was based on five mitotic metaphases. The chromosome

145   measurements were carried out using ImageJ software (27). The relative chromosome lengths

146   were calculated in each specimen independently as a percentage of the total chromosome

147   length (TCL) of the haploid set, including sex chromosome. Chromosome morphology was

148   classified according to Levan et al. (28).

149

150   Our study also includes detection of constitutive heterochromatin and nucleolus organizing

151   regions. Male mitotic plates were used to visualize these markers. Constitutive

152   heterochromatin was detected by C-banding following Král et al. (29). Preparations were

153   stained by 5% Giemsa solution in Sörensen phosphate buffer for 75 min. NORs were

154   visualized using fluorescence in situ hybridization (FISH) with a biotin-labeled probe for 18S

155   rDNA sequences. The probe was obtained from *Dysdera erythrina* Walckenaer, 1802

156   (Dysderidae). FISH, probe detection by streptavidin-Cy3 and signal amplification was

157   performed as described by Forman et al. (30).

158

159   *DNA extraction, amplification and sequencing*

160

161   We isolated the DNA from legs of three *A. snetsingeri* individuals. We washed the ethanol-

162   fixed legs twice for 15 min using 1 ml of 10 mM Tris-HCl (pH 7.5) with 5 mM EDTA.

163   Subsequently, we extracted the DNA using a NucleoSpin Tissue XS kit (Macherey-Nagel,

164   Düren, Germany) according to the manufacturer's instructions. We then amplified the DNA

165   using primers targeting nuclear (ITS2) and mitochondrial (CO1) loci using the following

166   polymerase chain reaction mix: 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1%

167   Triton X-100, 0.2 mM dNTP (each), 1 μM forward primer, 1 μM reverse primer, 0.5 U of Taq

168   DNA polymerase (Top-Bio, Prague, Czech Republic), and 300 ng of extracted genomic DNA.

169   The total reaction volume was 25 μl. To amplify the ITS2 locus, we used the primers

170   ApicITS2FW2 (5′-CGATGAAGAACGCAGCCAGCTGCGAG-3′; (31)) and RITS (5′-

171   TCCTCCGCTTATTGATATGC-3′; (32)). To amplify the CO1 locus, we used the primers

172   LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′; ((33)) and C1-N-2194 (5′-

173   CTTCTGGATGACCAAAAAATC-3′; (34)). We performed the reaction using an Eppendorf

174   Mastercycler Pro thermal cycler (Eppendorf, Hamburg, Germany) for 36 cycles with 15-s

175   denaturation at 94 °C, 2-min annealing at 43–57 °C, followed by a 1–3-min extension at 72

176   °C. We initiated the cycling with a 2-min denaturation at 94 °C and terminated it after 5-min

177   incubation at 72 °C. Subsequently, we purified the amplified DNA using USB Exo-SAP-IT

178   (Affymetrix, Santa Clara, CA) and bidirectionally sequenced the amplicons using an ABI

179   3130 DNA Analyzer (Applied Biosystems, Foster City, CA). For the three individuals of *A.*

180   *snetsingeri* analyzed in their ITS2 locus and two for their CO1 locus, all the obtained ITS2

181   and CO1 sequences were identical. The resulting consensus DNA sequences were submitted

182   to NCBI GenBank under accession numbers MT957000-MT957001 (CO1) and MT957146-

183   MT957148 (ITS2).

184

185   *Alignments and phylogenetic analyses*

186

187   We aligned the newly generated sequences with those of nine *Atypus* spp. obtained from

188   NCBI GenBank as of September 7, 2020, and sequences of the corresponding outgroups by

189   using MUSCLE (35,36) (gap opening penalty -400, gap extension penalty 0, clustering

190   method UPGMB, lambda 24). We manually corrected the alignments for any inconsistencies,

191   trimmed the aligned sequences to ensure that they all represent the same extent of the

192   analyzed locus, removed short-length sequences from the alignments, and used only trimmed

193   sequences for further analyses. The trimmed ITS2 locus [containing partial 5.8S ribosomal

194   DNA and partial (close to full-length) ITS2 sequences] corresponded to nt 62-385 (324 bp) of

195   *Atypus baotianmanensis* Hu, 1994 KP208877.1. The trimmed CO1 locus (partial CO1 coding

196   sequence) corresponded to nt 23-595 (573 bp) of *Atypus piceus* KX536935.1. For each locus,

197   we calculated the maximum likelihood fits of 24 nucleotide substitution models. We used a

198   bootstrap procedure at 1,000 replicates and the nearest-neighbor-interchange as the maximum

199   likelihood heuristic method to determine the tree inference when the initial tree was formed

200   using a neighbor joining algorithm. We used best-fit models for the maximum likelihood

201   phylogenetic analyses, including the estimates of evolutionary divergence between sequences.

202

203   **Results**

204

205   *Phylogenetic analysis*

206

207   Analysis of the DNA of *A. snetsingeri* has clarified its identity and the unusual presence of the

208   genus in North America. We found that the CO1 locus (Fig. 6A) had a 100% sequence

209   similarity (genetic distance of zero) with the matching 639bp-long CO1 locus of *A. karschi*

210   (SDSU_MY4706) from the Honshu island, Japan (Hedin et al. 2019). After *A. karschi* the

211   most closely related species for which sequences were available was the Asian *Atypus*

7

212 *heterothecus* Zhang, 1985, with a genetic distance of 0.131 ± 0.021 of base substitutions per

213 site between sequences. The European species, *Atypus piceus* and *Atypus affinis*, were basal to

214 *A. snetsingeri* as well as to the whole group of hitherto sequenced Asian *Atypus* spp. (Fig.

215 6A). Concerning the ITS2 locus, the sequences of only two other *Atypus* spp. are known (Fig.

216 6B); therefore, this hypervariable locus awaits future analyses when more comparative data

217 are available. The genetic distance to the closest species already sequenced in the ITS2 locus,

218 *Atypus baotianmanensis*, was 0.109 ± 0.022 of base substitutions per site between the

219 sequences.

220

221 **Figure 6.** Phylogenetic analyses of the position of *Atypus karschi* (Pennsylvania, USA) in the

222 genus *Atypus* based on the sequences of the CO1 (**A**) and ITS2 (**B**) loci by the maximum

223 likelihood approach. The evolutionary history was inferred using the Tamura-Nei model (**A**)

224 or the Kimura 2-parameter model (**B**), both with a discrete Gamma distribution used to model

225 evolutionary rate differences among sites. The models were selected based on the highest

226 Bayesian information criterion scores of the maximum likelihood fits. The trees are drawn to

227 scale, with branch lengths indicating the number of substitutions per site. All codon positions,

228 including noncoding positions, were included; the analyses were based on 573 positions (**A**)

229 or 345 positions (**B**).

230

231 *Taxonomy*

232

233 Based on an exact match of the genetic CO1 barcode data, the *Atypus snetsingeri* purse-web

234 spiders in Pennsylvania appear to represent an introduced local population of the Asian

235 species *Atypus karschi*. In the remainder of this paper those spiders are referred to as *Atypus*

236 *karschi* 'from Pennsylvania'. The specific epithet *snetsingeri* is relegated to a junior synonym

237 of *karschi*.

238

239 **Atypus karschi** Dönitz, 1887

240 *Atypus snetsingeri* Sarno, 1973: Sarno 1973 (21): page 38, figs 1–9 (description of both

241 sexes). **New synonymy.**

242 *A. snetsingeri* Gertsch and Platnick 1980 (23): page 11, figs 9, 13–20 (both sexes).

243 *A. snetsingeri* Schwendinger 1990 (7): page 360, fig. 18 (female).

244

8

245 *Remarks.* The synonymy was based on finding that the CO1 gene, used as a molecular
246 barcode, of *snetsingeri* specimens from Pennsylvania was identical with that of *Atypus*
247 *karschi* from the Honshu island, Japan (37).

248

249 *Karyotype*

250

251 The female karyotype of *A. karschi* from Pennsylvania showed 2n = 42 chromosomes and the
252 male had 2n = 41 (Fig. 5A), suggesting an X0 sex chromosome system. Chromosomes were
253 metacentric except for one pair, which exhibited submetacentric morphology (Fig. 5B). The
254 chromosome pairs gradually decreased in size, with the length of chromosome pairs in the
255 male ranging from 7.13% to 3.31% of TCL and in the female from 6.13% to 3.31% of TCL.
256 The sex chromosome was a metacentric element of medium size in both male (TCL = 4.27%)
257 and female (TCL = 4.09%) (Fig. 5A, B). Concerning meiosis, pachytene nuclei were found in
258 both the male and female specimen. In the male pachytene, the univalent X chromosome was
259 on the periphery of the nuclei. X chromosome arms were often associated with each other
260 during this period. Moreover, the X chromosome showed positive heteropycnosis (i.e., it was
261 stained more intensively than other chromosomes). The other bivalents exhibited prominent
262 knobs (Fig. 5C).

263

264 **Figure 5.** Chromosomes of *Atypus karschi*, Pennsylvania, USA. **A, B.** Male (A) and female
265 karyotypes (B), stained by Giemsa, based on mitotic metaphase. 2n♂ = 41, X0; 2n♀ = 42,
266 XX. Empty arrowhead – centromere of submetacentric pair. **C.** Male pachytene. Note
267 heterochromatic X chromosome on the periphery of the nucleus and prominent knobs on the
268 bivalents. Inset: scheme of sex chromosome. Note an association of X chromosome arms.
269 Arrow – sex chromosome. **D.** Male mitotic metaphase, C-banding. Chromosomes exhibit
270 intercalar and terminal heterochromatin blocks. Inset: magnified submetacentric chromosome
271 containing a large block of heterochromatin (from another mitotic metaphase). Arrowhead – a
272 large block of heterochromatin, empty arrowhead – centromere. **E.** Male mitotic metaphase,
273 detection of rDNA cluster (FISH). Note chromosomes of a submetacentric pair containing a
274 terminal rDNA cluster at long arm. Arrowhead – rDNA cluster, empty arrowhead –
275 centromere. Scale bars: 10 μm.

276

277 C-banded chromosomes exhibited small intercalar and terminal blocks of heterochromatin.
278 The submetacentric pair showed a prominent large block at the terminal part of the long arm

9

279   (Fig. 5D). It occupied on average 36% of the chromosome length (n = 10). The karyotype

280   contained one NOR locus that was localized in the end of the long arm of the submetacentric

281   pair (Fig. 5E). The NOR colocalised with the large block of heterochromatin and was of

282   considerable size (37.2% of the chromosome length, n = 8).

283

284   *Habitat*

285

286   The eight *Atypus karschi* sites that we visited in Delaware County in 2013 represented

287   suburban neighborhoods, small wooded parks, narrow riparian zones along developed stream

288   corridors, and protected parklands (Appendix 1). The purse-webs were located in a variety of

289   habitats at those sites, including the shrubs along suburban sidewalks, slopes and bottoms of

290   wooded valleys, beech forests and a fallow field that is mowed annually. Typical habitats of

291   *A. karschi* in Pennsylvania are shown in Fig. 1.

292

293   **Figure 1.** Habitats of *Atypus karschi* in Pennsylvania, USA; (1A) suburban bushes along Ellis

294   Ave (~200 m from type locality of *Atypus snetsingeri*), (1B) fallow field at Tyler Arboretum,

295   (1C) riparian woods, Swedish Cabin site on Darby Creek, (1D) forest, Smedley Park.

296

297   The inclination (slope) of the sites varied from 0–40°, ranging from a flat field to riparian

298   hillsides. Where a site in our study had a slope it usually faced the south but the azimuth of

299   orientation varied from 95–340°, excluding only the coldest north and north-east exposures.

300   The soil on slopes was usually not aggregated, was sandy or powdery, and of yellow or grey

301   color below the shallow humus layer. In valley bottoms, the spider lived in fluvisol and in the

302   suburbs in anthropogenic soils. Soil penetrability ranges from 0.5 to 3.25 (n = 14, mean 2.02

303   ± 0.31). The webs are typically associated with woody vegetation, and bush/shrub cover

304   ranged from 5–100 % (mean 40 %) and tree cover from 0–90 % (mean 50 %). The soil

305   surface where webs occurred was without moss, and the herbaceous cover was usually sparse

306   (from 0–90 %, mean 20 %).

307

308   *Natural history*

309

310   The above-ground webs we observed were vertical and mostly attached to the base of thin

311   stems of bushes or on trees (Figs 2, 3), but a few were attached to rock. In early November

312   three size categories were visually distinguished in the field by their relative web diameters,

10

313 representing small and medium juveniles, and adult females. According to prey remnants

314 found on their webs, they feed on millipedes (*Julida* and *Polydesmus* sp.), snails (*Cochlicopa*

315 sp.), woodlice (*Porcellio* sp.) and carabid beetles.

316

317 **Figure 2.** *Atypus karschi* and its webs in Pennsylvania, USA, (2A) adult female and male (on

318 the right), (2B) vertical web attached to the base of a tree and (2C) to a boulder, (2D)

319 horizontal web covered in thatch, (2E) thatch removed, (2F) view of trimmed ground in front

320 of bushes with 16 purse webs.

321

322 **Figure 3.** The distribution of *Atypus karschi* in Pennsylvania, USA. The peaks of the polygon

323 represent the outermost sites. The circles mark the sites described in this study. The black

324 circle is the type locality of *Atypus snetsingeri*.

325

326 Eleven out of 18 adult female webs that were excavated contained juveniles. There was no

327 significant difference between the body size (carapace length) of the females with (n = 11)

328 and without (n = 7) juveniles (all females: n = 18, min 5.04 mm, max 6.18 mm, mean 5.68 ±

329 0.09 mm) (Welch two sample *t*-test t = 1.45, *p* = 0.17). The number of juveniles ranged from

330 70 to 201 (n = 10, mean 121.30 ± 11.66) and did not correlate with the body size of the female

331 (Pearson's correlation n = 10, r = 0.32, *p* = 0.37). The length of the subterranean section of

332 tube associated with the burrow ranged from 6 to 10 cm (n = 13, mean 8.3 ± 0.3 cm) and did

333 not correlate with the body size of the spider (Pearson's correlation, n = 13, r = -0.44, *p* =

334 0.13). The length of the above-ground tube ranged from 5 to 13 cm (n = 14, mean 8.54 ± 0.67

335 cm) and also did not correlate with the body size of the spider (Pearson's correlation, r = 0.02,

336 *p* = 0.94). The length of the above-ground purse-web was more variable than the length of its

337 subterranean part (F test, n = 13, F = 0.23, *p* = 0.018) (Fig. 4). The ratio of below-

338 ground/above-ground lengths ranged from 0.62 to 1.80 (n = 14, mean 1.09 ± 0.08) and did not

339 correlate with the body size of the spider (Pearson's correlation, r = 0.02, *p* = 0.94).

340

341 **Fig. 4.** Boxplots showing the variation of the below-ground and above-ground lengths of

342 excavated purse-webs of *Atypus karschi*, Pennsylvania, USA (n = 13). The means are

343 indicated by an x and the hollow dot indicates an outlier (less than the 25th percentile minus

344 1.5 × Interquartile range).

345

346 **Discussion**

11

347

348 *Genetic identity of Atypus snetsingeri*

349

350 The presence of a geographically isolated population of an *Atypus* species in North America,

351 where the native purse-web spiders are in the genus *Sphodros*, has been mildly controversial.

352 Due to the species' location on the eastern coast of the USA a close relationship with

353 European *Atypus* species could have been expected. However, morphologically, *A. snetsingeri*

354 was known to closely resemble the Asian *A. karschi* (7,23). Raven (24) hypothesized that the

355 single *Atypus* species in the USA was introduced by man.

356

357 The newly obtained molecular data for *A. snetsingeri* have resolved those questions by

358 showing that the Pennsylvania species is more closely related to Asian species of *Atypus* than

359 to European species. More specifically, *A. snetsingeri* was a genetic match with sequence data

360 for *A. karschi* from Japan, affirming that the species represents an East Asian introduction.

361 Based on these data we propose a formal synonymy for *A. snetsingeri*, which now becomes a

362 junior synonym of *Atypus karschi*. Differences reported for morphological features compared

363 to *A. karschi* in Asia probably represent intraspecific variation given the small number of *A.*

364 *snetsingeri* specimens actually examined by researchers (7,23).

365

366 Parts of the genome of "*Atypus snetsingeri*" (based on NCBI sequences DQ639853.1,

367 DQ680323.1 and KY016940.1) were used in spider phylogeny studies to represent the genus

368 *Atypus* (37–39) or the entire family Atypidae (40). Wheeler et al. (1) used *A. snetsingeri* and

369 *A. affinis* data to represent *Atypus*, and added *Sphodros* for the family Atypidae. Recently the

370 entire mitochondrial genome was sequenced for *Atypus karschi* in China (41), which is highly

371 useful for further comparative studies of the Atypoidea.

372

373 *Karyology*

374

375 Most karyotype data on spiders concerns araneomorphs (42), but some karyotypes of

376 mygalomorph spiders have been published (17,18,20,43,44). Representatives of the

377 superfamily Atypoidea display a similar range of diploid numbers as araneomorph spiders

378 (from 14 to 47). Most Atypoidea also exhibit the X0 sex chromosome determination system,

379 which may be the ancestral characteristic sex chromosome determination of this superfamily

380 (20).

381

382   In the family Atypidae only four species in the genus *Atypus* have been studied

383   cytogenetically. *Atypus karschi* in this study exhibits $2n\male = 41$, X0 and predominance of

384   metacentric chromosomes, which is in accordance with the karyotypes of central European

385   species *A. piceus* and *A. muralis* (18). These karyotype features could be ancestral within the

386   genus *Atypus*. The karyotype of European *A. affinis* having $2n\male = 14$, XY, was derived from

387   chromosomal complement $2n\male = 41$, X0 by series of chromosomal fusions leading to

388   decreasing of diploid count and formation of a neo-sex chromosome system XY (18).

389

390   Remarkably, an earlier karyotype developed for *A. karschi* in Japan (19) differs considerably

391   from those reported in this study from Pennsylvania. The karyotype reported from Japan

392   consisted of approximately of 44 acrocentric chromosomes, including an X_1X_20 system, not

393   the 42 chromosomes and X0 pattern reported here. The discrepancy may be due to

394   interpopulation variability, but although mygalomorph spiders exhibits considerable

395   karyotype diversity (20), such an enormous degree of interpopulation karyotype variability is

396   very unlikely. Therefore, we suggest that the karyotype data of the Japanese population may

397   have been misinterpreted. The karyotype of *Atypus* is formed by relatively high number of

398   small chromosomes, which makes it difficult to determine the precise diploid number and

399   chromosome morphology. Moreover, the method of chromosome preparation used by Suzuki

400   (19) did not include treatment with a hypotonic solution, so the spreading of chromosomes

401   would have been less pronounced than in the present study using the methodology of Král et

402   al. (20). Regarding determination of the sex chromosome system, a single metacentric X

403   chromosome of an X0 system could be erroneously considered as two acrocentric X

404   chromosomes of an X_1X_20 system attached at one end during meiosis.

405

406   Within the framework of our cytogenetic analysis we were able to detect constitutive

407   heterochromatin for the first time in the Atypoidea. Most chromosomes of *A. karschi*

408   exhibited intercalar and terminal blocks of heterochromatin. The distribution of blocks

409   suggests that 1) most intercalar blocks are placed at centromeric regions and 2) terminal

410   blocks are formed at telomeric regions. This is consistent with the pattern of constitutive

411   heterochromatin distribution most commonly found in spiders (43).

412

413   Nucleolus organizer regions (NORs) are chromosome domains comprised of tandemly

414   repeated sequences of rRNA genes that aid formation of the nucleolus after division (45), and

13

415 their location on chromosomes may have taxonomic value. These regions have been detected

416 in ten species of mygalomorphs including four species of Atypoidea ((20), this study). The

417 number of NORs in Atypoidea ranges from one to four loci, and they are always situated on

418 chromosome pairs. NORs are usually detected by impregnation with silver nitrate or by

419 fluorescent in situ hybridization (FISH) with rDNA probe, although the first technique can

420 underestimate absolute number of NORs by visualizing only loci transcribed during previous

421 cell cycle (46). However, most NOR detections in mygalomorphs have been performed by

422 silver staining. Fluorescence in situ hybridization, which we applied to detect NORs of *A.*

423 *karschi* in this study, have been used with only one other mygalomorph species, *Tliltocatl*

424 *albopilosum* Valerio, 1980 (Theraphosidae) (20). Both species display one terminal NOR

425 localized on a chromosome pair, which may be the ancestral condition for spiders (20). The

426 NOR of *A. karschi* is associated with heterochromatin, which is a common feature of rDNA in

427 eukaryotes (e.g., (47,48)). Comparison of the length of the rDNA cluster and heterochromatin

428 block suggests that heterochromatin associated with the NOR is formed by inactivated rDNA.

429 This pattern is in an agreement with the current model for NOR organization, in which major

430 regions of rDNA are often inactivated and only a restricted fraction of rDNA is transcribed

431 (49).

432

433 *Habitat*

434

435 *Atypus karschi* in Pennsylvania appears to be undemanding regarding habitat requirements

436 (see Appendix 1) and can be locally abundant where it occurs. The webs are built in soil of

437 varied humidity and physical parameters and are associated with a variety of supports (trees,

438 shrubs, grasses, rock, walls and fences) over a ground surface either covered by or nearly

439 devoid of litter. Webs were found on flat terrain and on slopes of various inclinations and

440 orientation. In Pennsylvania it occurs in wooded areas but is also reliably found in some

441 suburban neighborhoods, where webs are built at the base of shrubs or along walls and fences.

442 Miyashita (50) reported a very similar situation in Japan where *A. karschi* is "common" and

443 "usually live(s) in shady and humid places such as woods and shrubberies." Images posted on

444 iNaturalist (51) of *A. karschi* in East Asia also support a tolerance of human-modified settings

445 where they were encountered (wall, fence and stone garden).

446

447 In sharp contrast, European *Atypus* species usually require very specific edaphic conditions

448 and are associated with specific vegetation types and sun-facing slopes (14). Unlike *A. karschi*

449   in Pennsylvania, they are not found in habitats subject to recent or regular disturbance and are

450   uncommon enough to be red-listed in all Central European countries. Their presence at a site

451   is an indicator of a relic habitat worthy of conservation management (8,52).

452

453   *Natural history*

454

455   The life history of *A. karschi* in Japan was studied in detail by Miyashita (50) under semi-

456   outdoor conditions and reported with prior data from Aoki (53) and Yaginuma (54). Basic

457   natural history parameters of *A. karschi* in eastern Asia and *A. snetsingeri* in the USA are

458   contrasted in Table 1 and indicated a similarity in every respect (body size, ontogeny,

459   phenology, fecundity, morphology of webs, environment). No difference was found that

460   would refute the conspecificity of the Pennsylvania population with Asian *A. karschi*.

461

462 **Table 1.** Natural history parameters (body size, ontogeny, phenology, fecundity, morphology

463 of webs, environment) reported for *Atypus karschi* in Japan and for the introduced population

464 known as *A. snetsingeri* in Pennsylvania, USA.

465

Natural history parameter	*Atypus karschi* - Japan	*Atypus snetsingeri* - USA
Body size		
Carapace length of males	3.87–4.23 mm (55)	3.2–4.6 mm (21)
Carapace length of females	4.77–5.76 mm (55)	3.4–7.0 mm (21)
Ontogeny		
No. of eggs	mean 124, maximum 270 (50)	mean 121, maximum 201 (this study)
No. of moults before reaching maturity	8–9 in males, 9–11 in females (50)	unknown
Age of maturation	3 years (50)	possibly 3 years, based on three concurrent web size categories in the population (this study)
Phenology		
Mating season	June – (July) August (50,53)	June–August (21,22)
eggs	July (August) - September (50,53,54)	July-September (22)
larvae	October (56,57)	September (22)
1st nymphal instar	late October–April (dispersion) (50,53,53)	September–March (dispersion) (22)
Morphology of webs		
Orientation of the capturing tube	vertically attached to the tree trunk or rock (57)	vertically attached to the tree, hedge or wall (21) or horizontally oriented in grass and thatch (22)

16

Length of the capturing tube	Up to 20 cm (almost the same as the depth of the burrow) (57)	Up to 25 cm (21)
Depth of the burrow	Up to 20 cm (50,57)	Up to 20 cm (21)
Environment		
Microclimate	Shady and moist, in the forest close to the trees, rocks or bamboo (50,57)	Mostly shady and moist, in litter and areas with loose soil (this study)
Habitat	Forests and shrubs (50,57)	Forests and shrubs, disturbed areas (this study)

466

467

468 Although Gertsch and Platnick (23) contemplated whether the above-ground purse-web
469 orientation could be useful to distinguish between *Atypus* (horizontal tubes) and *Sphodros*
470 (vertical tubes), species in both genera can and do make both kinds of webs (58). In our study
471 we observed only vertical webs of *A. karschi* at the sites visited, but the spiders are known to
472 make horizontal webs in thatch and grass (22). In Tyler's fallow field, for example, vertical
473 webs can be found on plant stems within a few centimeters of horizontal webs in surrounding
474 grasses. While vertical tubes are characteristic of North American *Sphodros* species, *Sphodros*
475 *niger* Hentz, 1842 may preferentially build horizontal tubes, at least in some settings (59,60).
476 Mckenna-Foster et al. (61) found that *Sphodros rufipes* Latreille, 1829 in New England will
477 use whatever support is available and many webs were close to the ground. The suggestion
478 that horizontal webs are an adaptation to prey capture under the snow (7) may ignore the
479 function of vertical webs at ground level. In Pennsylvania *A. karschi* habitats experience snow
480 and cold temperatures each year. In Tyler's field the horizontal tubes laying near the soil
481 surface tend to be well-buffered by leaf litter or thatch, but basal sections of vertical webs are
482 similarly buffered and may likewise function normally in a subnivean environment when both
483 prey and spiders are active (Tessler, personal observations).

484

485 In this study we measured the webs of fourteen adult females from a fallow field with
486 homogeneous soil. We found the overall length of the webs were shorter than those observed
487 by Sarno (21) around a house foundation and on shrubs in a suburb (see Table 1), probably

17

488   reflecting different conditions and prey availability between sites. The length of the aerial

489   tube was more variable than that of the underground part (Fig. 4). Less variation in the

490   underground web length may reflect a minimum depth of the burrow necessary for suitable

491   microclimate, constraints imposed on digging, or the shallow soil frost depth in winter. Depth

492   of burrows differs among European *Atypus* species, where the species living in arid habitats

493   tend to dig deeper burrows than those living in woody vegetation (8).

494

495   Concerning the number of juveniles found within maternal webs, *A. karschi* in Pennsylvania

496   (max. 201 juveniles) has a similar number as *A. karschi* in Asia. Likewise, European *Atypus*

497   species also have large broods (*A. affinis* max. 191, *A. piceus* max. 168, *A. muralis* max. 150;

498   M. Řezáč, personal observations).

499

500   Prey we observed for *A. karschi* in Pennsylvania were mostly ground-based invertebrates and

501   favored millipedes, similar to observations on *S. niger* in New England (60). *Atypus karschi* in

502   Pennsylvania has also been observed feeding on earthworms, and will readily capture

503   orthopteroids and other insects that contact the web while climbing vegetation, including the

504   pestiferous spotted lanternfly (Hemiptera: Fulgoridae: *Lycorma delicatula* White, 1845) that

505   was recently introduced into Pennsylvania from Asia (Tessler, personal observations).

506

507   *Range of* Atypus karschi *in Pennsylvania*

508

509   *Atypus karschi* seems to possess several preadaptations that allowed it to successfully

510   colonize southeastern Pennsylvania following its introduction. First, it occurs over a wide area

511   in eastern Asia with a similar climate (Japan (57); Chinese provinces Hebei, Anhui, Sichuan,

512   Guizhou, Hubei, Hunan, Fujian (55); Taiwan (62); Korea's Ungil Mountain (63)). Second, it

513   produces a large number of lightweight juveniles that are capable of aerial dispersal

514   (22,50,64). Third, the species in Pennsylvania is ecologically plastic and does not appear to

515   have specific edaphic or microclimatic requirements, even thriving in settings frequently

516   impacted by humans.

517

518   The original description and first review of the species *A. snetsingeri* in Pennsylvania was

519   based on specimens taken from two nearby suburban sites in Lansdowne and Upper Darby in

520   eastern Delaware County near Philadelphia (21,23). At that time, it was known be common

521   and unnoticed in the surrounding areas within the Cobbs Creek and Darby Creek drainage

522   basins (Tessler, personal observations). It has subsequently been sought and found in many

523   (not all) of the forested riparian zones and wooded parklands across Delaware County and

524   also at sites in adjacent areas of Philadelphia, Montgomery, Chester and Berks counties (Fig.

525   Map). Many neighboring areas, including most of urban Philadelphia, remain unexplored

526   (22). A few of those species determinations were based finding males, but the majority

527   involved excavating a web to extract the spider and examine the sternum sigilla pattern and

528   the posterior lateral spinnerets (PLS) to distinguish it from *Sphodros* species (23). In

529   particular, *A. snetsingeri* has a distinctly four-segmented PLS, whereas the northern *Sphodros*

530   species have only three segments (*S. niger, S. rufipes, S. atlanticus*).

531

532   Spiderlings of *A. karschi* in Pennsylvania use silk for aerial dispersal in the spring (22), which

533   may have contributed to expanding its range from an original introduction locus. However,

534   the association of these spiders with highly developed land and disturbed habitats suggests a

535   wider transport opportunity via trees, woody shrubs and mulch moved within the region by

536   landscaping and nursery industries (Tessler, personal observations).

537

538   Interestingly, *Sphodros* purse-web spiders are also found in Pennsylvania and adjacent states

539   (23), but none have ever been reported in the same areas as *A. snetsingeri*. This is

540   unsurprising because atypids and their webs are rarely noticed or reported even when they are

541   locally abundant (22,65). Sightings of wandering *Sphodros* males reported in iNaturalist (66)

542   indicate that *S. niger* is found in Pennsylvania west and north of the *A. karschi* area and

543   southward in neighboring states, and that *S. rufipes* occurs in Maryland and New Jersey south

544   and east of the Philadelphia area and northward into coastal New England. While perhaps

545   provocative, those observations are not evidence of displacement of any local species by the

546   introduction of *A. karschi*.

547

548   It is unlikely that the source and timing of *A. karschi*'s introduction to Pennsylvania will ever

549   be determined. The species has a broad native range in East Asia extending from China and

550   Taiwan to Japan (12), and it was recently reported in Korea (63). The Philadelphia region

551   (including Delaware County) has had a 300 year history of trade with East Asia that may have

552   included countless opportunities for accidental importation of a soil-associated spider among

553   potted plants. Indeed, Nentwig (67) suggests that spiders introduced with potted plants have

554   higher establishment rates relative to those introduced by other means. In the 1700s and 1800s

555   Philadelphia was the center of American botany and horticulture and many plants from

19

556 around the world, including Asia, were actively collected, imported and traded for exhibition
557 and cultivation in public and private gardens (68,69). Many of the region's great gardens and
558 arboreta of that era still exist to some extent (70), including Tyler Arboretum (visited in this
559 study) and Bartram's Garden in west Philadelphia, the home of noted American botanists
560 John Bartram and his son William (71,72). William Bartram's contemporary in the late 1700s,
561 William Hamilton, built his estate "The Woodlands" overlooking Philadelphia's Schuylkill
562 River and his gardens and greenhouse boasted of having every rare plant he'd ever heard of
563 from around the world (73,74). In 1784, after the American Revolution, direct shipping trade
564 began between Philadelphia and China and at its peak represented about a third of all US
565 trade with China (75). A very significant Asian botanical importation event occurred later, in
566 1926, when the Japanese government presented 1,600 flowering trees to the City of
567 Philadelphia to celebrate the 150th anniversary of American independence (76). Regarding
568 introductions of other soil-associated invertebrates, Asian jumping worms (*Amynthas* and
569 *Metaphire* spp.) were presumably brought to the US in the 1800s in the soil of potted plants,
570 and recent studies have shown that they displace native worms and are changing the soil
571 where they occur (77). Coincidentally, nonnative jumping worms are present at many *A.*
572 *karschi* sites in Pennsylvania (Tessler, personal observations).

573

574 *Conclusion*

575

576 Many spider species have been accidentally introduced by humans to a new continent and
577 became established (67), nearly all from the phylogenetically recent infraorder
578 Araneomorphae. Within the more primitive mygalomorphs, the Mexican redrump tarantula
579 (Theraphosidae) native to Mexico and Central America has become established in Florida
580 USA (78). Presumably escaped from the pet trade, these tarantulas dig burrows and appear to
581 be restricted to a small area with climate and habitat features similar to its native range. In this
582 study we show that *Atypus snetsingeri* in Pennsylvania is genetically conspecific with *Atypus*
583 *karschi* native to East Asia. The species appears to have been introduced by humans to
584 Pennsylvania, probably in association with potted plants, and is now naturalized and locally
585 common within a limited range that includes urban and forested areas. It is unlikely that the
586 source or timing of the introduction can be determined in a region renowned for its colonial-
587 era horticulturalists, elaborate international gardens, and long history of shipping trade with
588 East Asia. This is the first case of an introduced species of Atypoidea from the infraorder
589 Mygalomorphae.

590

1. Wheeler WC, Coddington JA, Crowley LM, Dimitrov D, Goloboff PA, Griswold CE, et al. The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. Cladistics. 2017;33(6): 574–616.

2. Enock F. The life-history of *Atypus piceus*, Sulz. Trans R Entomol Soc Lond. 1885;33(4): 389–420.

3. McCook HC. Nesting habits of the American purseweb spider. Proc Acad Nat. 1888;40: 203-220. Available from: https://www.biodiversitylibrary.org/bibliography/6885

4. Poteat WL. A tube-building spider. Notes on the architectural and feeding habits of *Atypus niger* Hentz. J Elisha Mitchell Sci Soc. 1890;6(2): 132–47.

5. Platnick NI. Spiders of the world: A natural history. London: Princeton University Press; 2020.

6. Bristowe WS. World of spiders. Collins; 1958.

7. Schwendinger PJ. A synopsis of the genus Atypus (Araneae, Atypidae). Zoologica Scripta. 1990;19(3): 353–66.

8. Řezáč M, Heneberg P. Conservation status of the only representative of infraorder Mygalomorphae (Araneae) in cultivated regions of Central Europe. J Insect Conserv. 2014;18(4): 523–37.

9. Coyle FA. Aerial Dispersal by Mygalomorph Spiderlings (Araneae, Mygalomorphae). J Arachnol. 1983;11(2): 283–6.

623　10.　Coyle FA, Greenstone MH, Hultsch A-L, Morgan CE. Ballooning Mygalomorphs: Estimates of the
624　　　　Masses of Sphodros and Ummidia Ballooners (Araneae: Atypidae, Ctenizidae). J Arachnol.
625　　　　1985;13(3): 291–6.

626　11.　Nentwig W, Blick T, Bosmans R, Gloor D, Hänggi A, Kropf C. Spiders of Europe. Version 5; 2021
627　　　　[cited 20 May 2021]. Database [Internet]. Available from: https://www.araneae.nmbe.ch

628　12.　World Spider Catalog. World Spider Catalog; 2021 [cited 30 Mar 2021]. Natural History Museum
629　　　　Bern. [Internet]. Available from: http://wsc.nmbe.ch

630　13.　Dippenaar-Schoeman AS, Jocqué R. African spiders: an identification manual. Pretoria: ARC-
631　　　　Plant Protection Research Institute; 1997.

632　14.　Řezáč M, Řezáčová V, Pekár S. The distribution of purse-web *Atypus spiders* (Araneae:
633　　　　Mygalomorphae) in central Europe is constrained by microclimatic continentality and soil
634　　　　compactness. J Biogeogr. 2007;34(6): 1016–27.

635　15.　Řezáč M, Tošner J, Heneberg P. Habitat selection by threatened burrowing spiders (Araneae:
636　　　　Atypidae, Eresidae) of central Europe: evidence base for conservation management. J Insect
637　　　　Conserv. 2018 Feb 1;22(1): 135–49.

638　16.　National Center for Biotechnology Information (NCBI). Query results for Atypidae genome data;
639　　　　2021 [cited 23 Oct 2021] Database [Internet]. Available from:
640　　　　https://www.ncbi.nlm.nih.gov/nuccore/?term=atypidae

641　17.　Král J, Kořínková T, Forman M, Krkavcová L. Insights into the Meiotic Behavior and Evolution of
642　　　　Multiple Sex Chromosome Systems in Spiders. CGR. 2011;133(1): 43–66.

643　18.　Řezáč M, Král J, Musilová J, Pekár S. Unusual karyotype diversity in the European spiders of the
644　　　　genus Atypus (Araneae: Atypidae). Hereditas. 2006;143(2006): 123–9.

645　19.　Suzuki S. Cytological studies in spiders. III. Studies on the chromosomes of fifty-seven species of
646　　　　spiders belonging to seventeen families, with general considerations on chromosomal
647　　　　evolution. J Sci Hiroshima Univ(B). 1954;15: 23–136.

648　20.　Král J, Kořínková T, Krkavcová L, Musilová J, Forman M, Herrera IMÁ, et al. Evolution of
649　　　　karyotype, sex chromosomes, and meiosis in mygalomorph spiders (Araneae: Mygalomorphae).
650　　　　Biol J Linn. 2013;109(2): 377–408.

651　21.　Sarno PA. New species of Atypus. Entomol News Philadelphia. 1973; 37-51.

652　22.　Tessler S. Map the Spider; 2021 [cited 2021 Mar 30]. Available from:
653　　　　http://www.mapthespider.com/

654　23.　Gertsch WJ, Platnick NI. A revision of the American spiders of the family Atypidae (Araneae,
655　　　　Mygalomorphae). Am Mus Novit; no. 2704; 1980. Available from:
656　　　　https://digitallibrary.amnh.org/handle/2246/5390

657　24.　Raven RJ. The spider infraorder Mygalomorphae (Araneae): cladistics and systematics. Bull Am
658　　　　Mus Nat. 1985;182. Available from: https://digitallibrary.amnh.org/handle/2246/955

659   25.   Srba M, Heneberg P. Nesting habitat segregation between closely related terricolous sphecid
660   species (Hymenoptera:Spheciformes): key role of soil physical characteristics. J Insect Conserv.
661   2012;16(4): 557–70.

662   26.   R Core Team. R: A language and environment for statistical   computing [Internet]. Vienna,
663   Austria: R Foundation for Statistical Computing; 2019. Available from: http://www.r-
664   project.org/

665   27.   Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nature
666   methods. 2012;9(7): 671–5.

667   28.   Levan A, Fredga K, Sandberg AA. Nomenclature for centromeric position on chromosomes.
668   Hereditas. 1964;52(2): 201–20.

669   29.   Král J, Kováč L, Šťáhlavský F, Lonský P, Ľuptáčik P. The first karyotype study in palpigrades, a
670   primitive order of arachnids (Arachnida: Palpigradi). Genetica. 2008;134(1): 79–87.

671   30.   Forman M, Nguyen P, Hula V, Král J. Sex Chromosome Pairing and Extensive NOR Polymorphism
672   in Wadicosa fidelis (Araneae: Lycosidae). CGR. 2013;141(1): 43–9.

673   31.   Heneberg P, Řezáč M. Two Trichosporon species isolated from Central-European mygalomorph
674   spiders (Araneae: Mygalomorphae). Antonie van Leeuwenhoek. 2013 Apr 1;103(4):713–21.

675   32.   White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA
676   genes for phylogenetics. PCR protocols: a guide to methods and applications. 1990;18(1): 315–
677   22.

678   33.   Folmer O, Black M, Wr H, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial
679   Cytochrome C oxidase subunit I from diverse metazoan invertebrates. Mol Mar Bio Biotechnol.
680   1994 Nov 1;3: 294–9.

681   34.   Bidegaray-Batista L, Macías-Hernández N, Oromí P, Arnedo MA. Living on the edge:
682   demographic and phylogeographical patterns in the woodlouse-hunter spider *Dysdera*
683   *lancerotensis* Simon, 1907 on the eastern volcanic ridge of the Canary Islands. Mol Ecol.
684   2007;16(15): 3198–214.

685   35.   Madeira F. mi Park Y. Lee J, Buso N, Gur T, Madhusoodanan N, Basutkar P, Tivey ARN, Potter SC,
686   Finn RD, et al. The EMBL-EBI Search and Sequence Analysis Tools APIs in 2019. Nucleic Acids
687   Res. 2019;47: W636-W641.

688   36.   National Center for Biotechnology Information (NCBI). Query results for Atypus genome data
689   [Internet]. 2021 [cited 2021 Jul 1]. Available from:
690   https://www.ncbi.nlm.nih.gov/nuccore/?term=Atypus%20[Organism]

691   37.   Hedin M, Derkarabetian S, Alfaro A, Ramírez MJ, Bond JE. Phylogenomic analysis and revised
692   classification of atypoid mygalomorph spiders (Araneae, Mygalomorphae), with notes on
693   arachnid ultraconserved element loci. PeerJ. 2019;7: e6864.

694   38.   Hedin M, Bond JE. Molecular phylogenetics of the spider infraorder Mygalomorphae using
695   nuclear rRNA genes (18S and 28S): conflict and agreement with the current system of
696   classification. Mol Phylogenet Evol. 2006;41(2): 454–71.

697 39. Bond JE, Hendrixson BE, Hamilton CA, Hedin M. A reconsideration of the classification of the
698   spider infraorder Mygalomorphae (Arachnida: Araneae) based on three nuclear genes and
699   morphology. PloS one. 2012;7(6): e38753.

700 40. Ayoub NA, Garb JE, Hedin M, Hayashi CY. Utility of the nuclear protein-coding gene, elongation
701   factor-1 gamma (EF-1γ), for spider systematics, emphasizing family level relationships of
702   tarantulas and their kin (Araneae: Mygalomorphae). Mol Phylogenet Evol. 2007;42(2): 394–409.

703 41. He A, Guo J, Peng H, Huang Z, Liu J, Xu X. The complete mitochondrial genome of *Atypus karschi*
704   (Araneae, Atypidae) with phylogenetic consideration. Mitochondrial DNA Part B. 2021;6(9):
705   2523–5.

706 42. Araujo D, Schneider MC, Paula-Neto E, Cella DM. The World spider cytogenetic database
707   [Internet]. 2020 [cited 2020 Jul 30]. Available from:
708   http://www.arthropodacytogenetics.bio.br/spiderdatabase/

709 43. Kořínková T, Král J. Karyotypes, sex chromosomes, and meiotic division in spiders. In: Spider
710   ecophysiology. Springer; 2013. p. 159–71.

711 44. Sember A, Pappová M, Forman M, Nguyen P, Marec F, Dalíková M, et al. Patterns of Sex
712   Chromosome Differentiation in Spiders: Insights from Comparative Genomic Hybridisation.
713   Genes. 2020;11(8): 849.

714 45. Clark MS, Wall WJ. Chromosomes: the complex code. [Internet]. London: Chapman & Hall Ltd;
715   1996 [cited 2021 Mar 29]. 345 p. Available from:
716   https://www.cabdirect.org/cabdirect/abstract/19960106700

717 46. Miller DA, Dev VG, Tantravahi R, Miller OJ. Suppression of human nucleolus organizer activity in
718   mouse-human somatic hybrid cells. Experimental Cell Research. 1976;101(2): 235–43.

719 47. Fujiwara A, Abe S, Yamaha E, Yamazaki F, Yoshida MC. Chromosomal localization and
720   heterochromatin association of ribosomal RNA gene loci and silver-stained nucleolar organizer
721   regions in salmonid fishes. Chromosome Res. 1998;6(6): 463–71.

722 48. Guetg C, Santoro R. Formation of nuclear heterochromatin. Epigenetics. 2012;7(8): 811–4.

723 49. Carmo-Fonseca M, Mendes-Soares L, Campos I. To be or not to be in the nucleolus. Nat Cell
724   Biol. 2000;2(6): E107–12.

725 50. Miyashita K. Postembryonic Development and Life Cycle of *Atypus karschi* DONITZ (Araneae:
726   Atypidae). 1992;10.

727 51. iNaturalist. Images of *Atypus karschi*. [Internet]. 2021 [cited 2021 Jul 28]. Available from:
728   https://www.inaturalist.org/observations?verifiable=true&taxon_id=360542

729 52. British Arachnology Society. Spider and Harvestman Recording Scheme website | Summary for
730   *Atypus affinis* (Araneae). [Internet]. 2021 [cited 2021 Jul 29]. Available from:
731   http://srs.britishspiders.org.uk/portal.php/p/Summary/s/Atypus+affinis

732 53. Aoki T. On the life cycle of Jigumo (*Atypus karschi* Dön.) in Wakajama city. Nanki-Seibutsu
733   Suppl. 1983;25: 43–8.

734 54. Yaginuma T. Spiders of Japan in Colour (enl, rev. ed.). Osaka: Hoikusha Publishing Co.; 1978.

735 55. Zhu M-S, Zhang F, Song D, Qu P. A revision of the genus Atypus in China (Araneae: Atypidae).
736   Zootaxa. 2006;1118(1): 1.

737 56. Bösenberg W, Strand E. Japanische spinnen. 1906.

738 57. Dönitz W. Uber die Lebensweise Zweier Vogelspinnen aus Japan. Sitzungsberichte der
739   Gesellschaft naturforschender Freunde zu Berlin. 1887;8–10.

740 58. Schwendinger PJ. On the genus Atypus (Araneae: Atypidae) in northern Thailand. Bull Br
741   Arachnol Soc. 1989;8(3): 89–96.

742 59. Beatty JA. Web structure and burrow location of *Sphodros niger* (Hentz) (Araneae: Atypidae).
743   Journal of Arachnology 14: 130-132. [Internet]. 1986 [cited 2021 Jul 21]. Available from:
744   https://www.americanarachnology.org/journal-joa/joa-all-
745   volumes/detail/article/download/JoA_v14_p130_grey.pdf

746 60. Edwards RL, Edwards EH. Observations on the natural history of a New England population of
747   *Sphodros niger* (Araneae, Atypidae). J Arachnol. 1990;29–34.

748 61. Mckenna-Foster A, Draney ML, Beaton C. An unusually dense population of *Sphodros rufipes*
749   (Mygalomorphae: Atypidae) at the edge of its range on Tuckernuck Island, Massachusetts. J
750   Arachnol. 2011;39(1): 171–3.

751 62. Saitō S. On the spiders from Tohoku (northernmost part of the main island), Japan. Saito Ho-on
752   Kai Museum Research Bulletin. 1939;18: 1–91.

753 63. Kim J, Cho Y. Unrecorded Korean *Atypus karschii* Doenitz (Atypidae Thorell, Genus Atypus).
754   Korean Arachnology. 2019;35 (1-2): 1–4.

755 64. Katsura K. A note on a ballooning of *Atypus karschi*. Atypus. 1975;64: 6.

756 65. Moler PE, Stevenson DJ, Mansell BW, Mays JD, Lee CW. Distribution and Natural History of
757   Purseweb Spiders, Sphodros spp. (Araneae: Mygalomorphae: Atypidae), in Florida, Georgia, and
758   Alabama. Southeastern Naturalist. 2020;19(4): 663–72.

759 66. iNaturalist. Map of the Genus Sphodros. [Internet]. 2021 [cited 2021 Jul 21]. Available from:
760   https://www.inaturalist.org/observations?place_id=any&subview=map&taxon_id=61444

761 67. Nentwig W. Introduction, establishment rate, pathways and impact of spiders alien to Europe.
762   Biol Invasions. 2015;17(9): 2757–78.

763 68. Chesney S. Encyclopedia of Greater Philadelphia | Botany. [Internet]. 2017 [cited 2020 Dec 14].
764   Available from: https://philadelphiaencyclopedia.org/archive/botany/

765 69. Sarudy B. Colonial and Early American Gardens: The Greenhouse in Early America [Internet].
766   2020 [cited 2021 Aug 24]. Available from:
767   https://americangardenhistory.blogspot.com/2018/12/the-greenhouse-conservatory-in-
768   early.html

769 70. Klein WM. Gardens of Philadelphia & the Delaware Valley. Temple University Press; 1995.

770 71. Rehder A. On the History of the Introduction of Woody Plants into North America. Arnoldia
771   6:13-23 [Internet]. 1946 [cited 2021 Jun 21]. Available from:

772   http://arnoldia.arboretum.harvard.edu/pdf/articles/1946-6—on-the-history-of-the-
773   introduction-of-woody-plants-into-north-america.pdf

774 72. O'Malley T. The Evidence of American Garden History. History of Early American Landscape
775   Design [Internet]. 2021 [cited 2021 Aug 24]. Available from:
776   https://heald.nga.gov/mediawiki/index.php?title=The_Evidence_of_American_Garden_History
777   &oldid=40049

778 73. Stetson SP. William Hamilton and His" Woodlands". The Pennsylvania Magazine of History and
779   Biography. 1949;73(1): 26–33.

780 74. Madsen K. To Make His Country Smile: William Hamilton's Woodlands. Arnoldia. 1989;49(2):
781   14–24.

782 75. Norwood D. The Encyclopedia of Greater Philadelphia | China Trade. [Internet]. 2016 [cited
783   2021 Jun 15]. Available from: https://philadelphiaencyclopedia.org/archive/china-trade/

784 76. Japan Society of Greater Philadelphia. Shofuso | History [Internet]. 2021 [cited 2021 Jul 15].
785   Available from: https://japanphilly.org/programs/festivals/subaru-cherry-blossom-
786   festival/about/history/

787 77. Qiu J, Turner MG. Effects of non-native Asian earthworm invasion on temperate forest and
788   prairie soils in the Midwestern US. Biological Invasions. 2017;19(1): 73–88.

789 78. Edwards GB, Hibbard K. The Mexican Redrump, *Brachypelma vagans* (Araneae: Theraphosidae),
790   an exotic tarantula established in Florida. Entomology Circular No. 394, May/June 1999, 2pp.
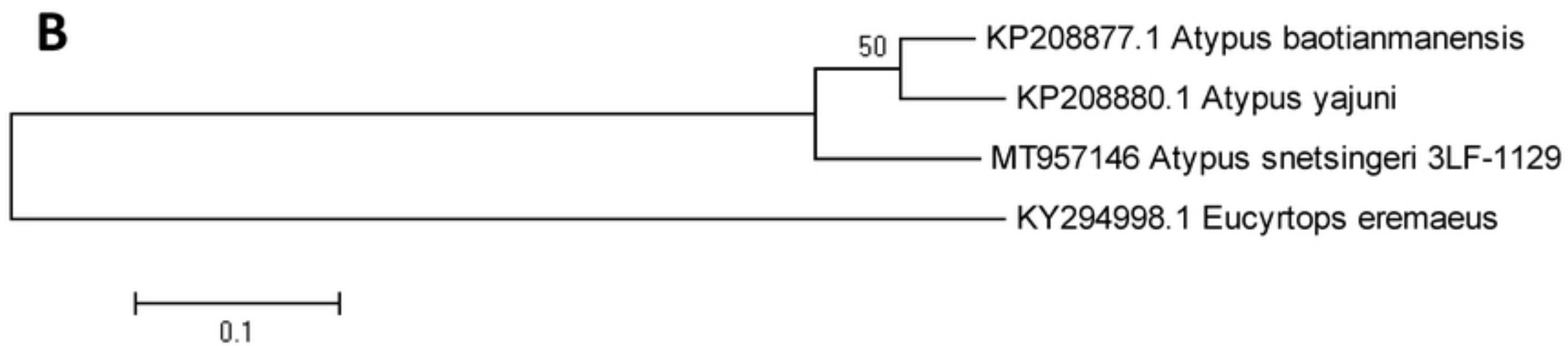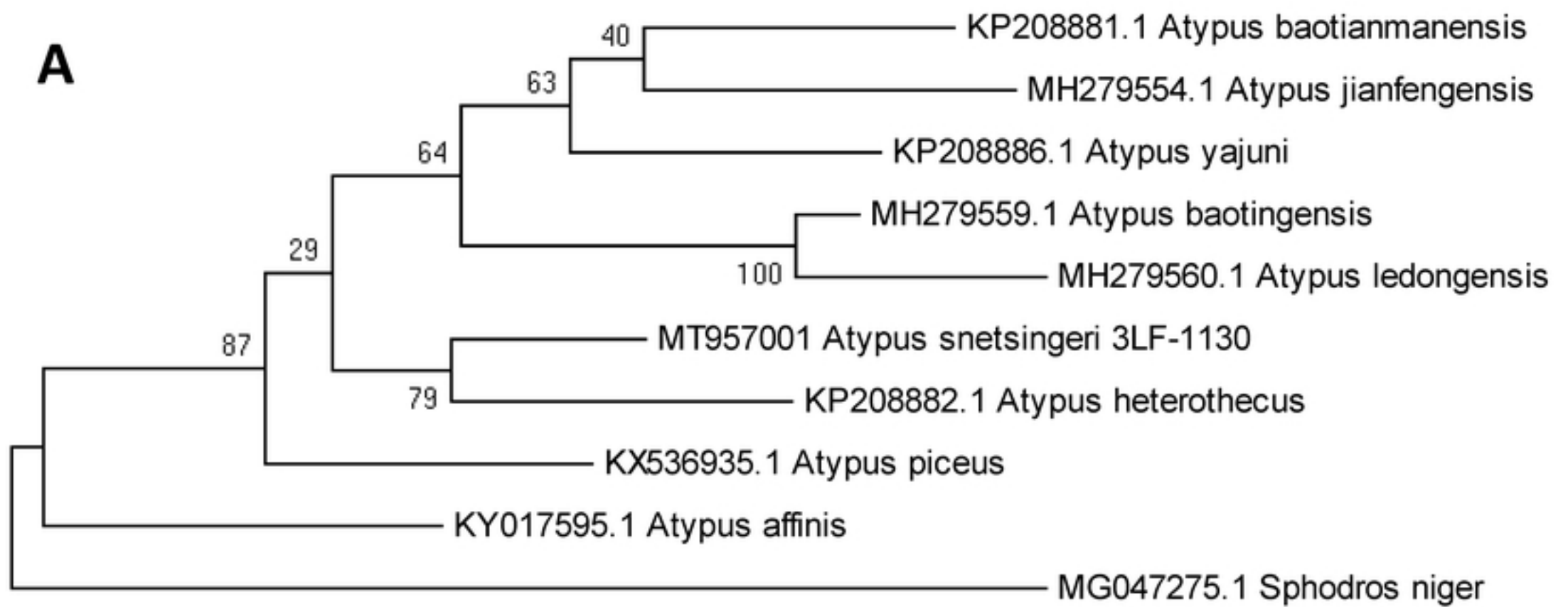791   [Internet]. 1999 [cited 2021 Jul 19]. Available from:
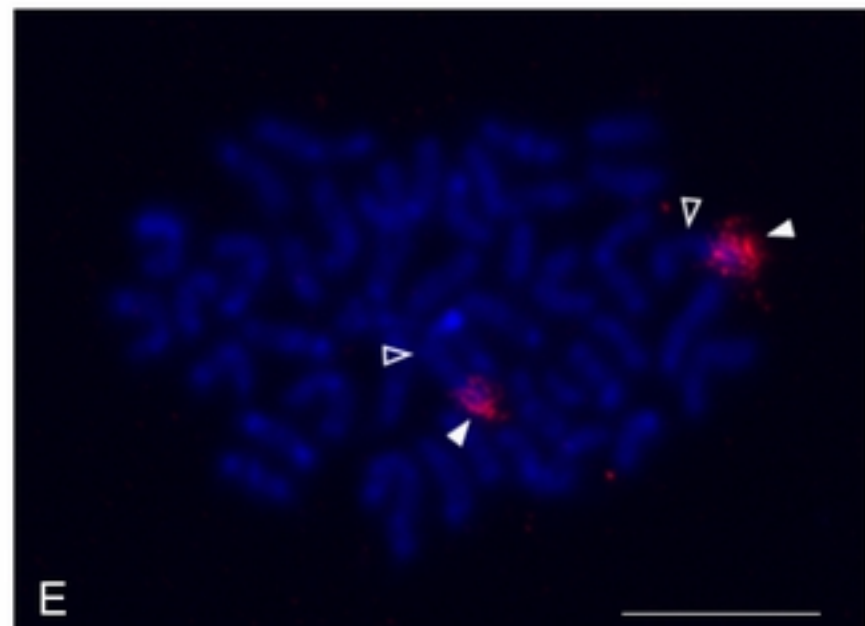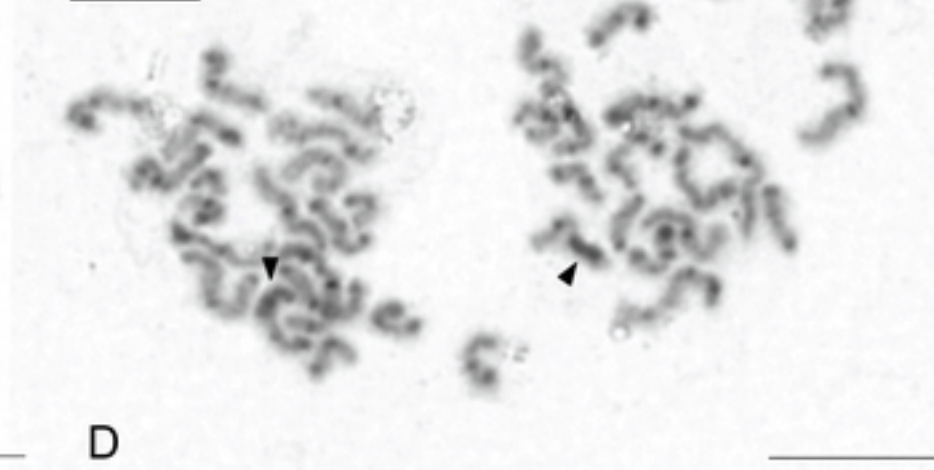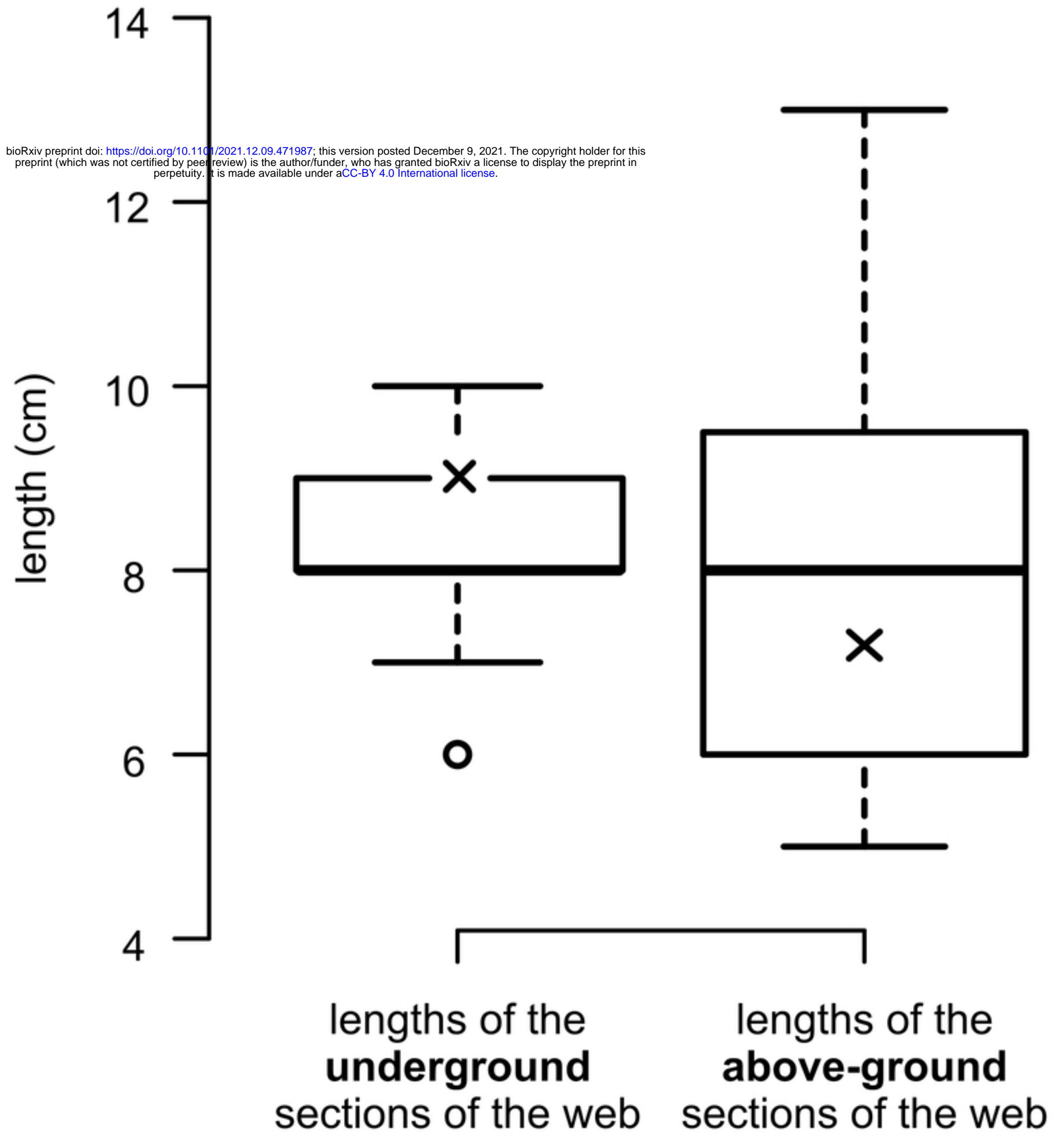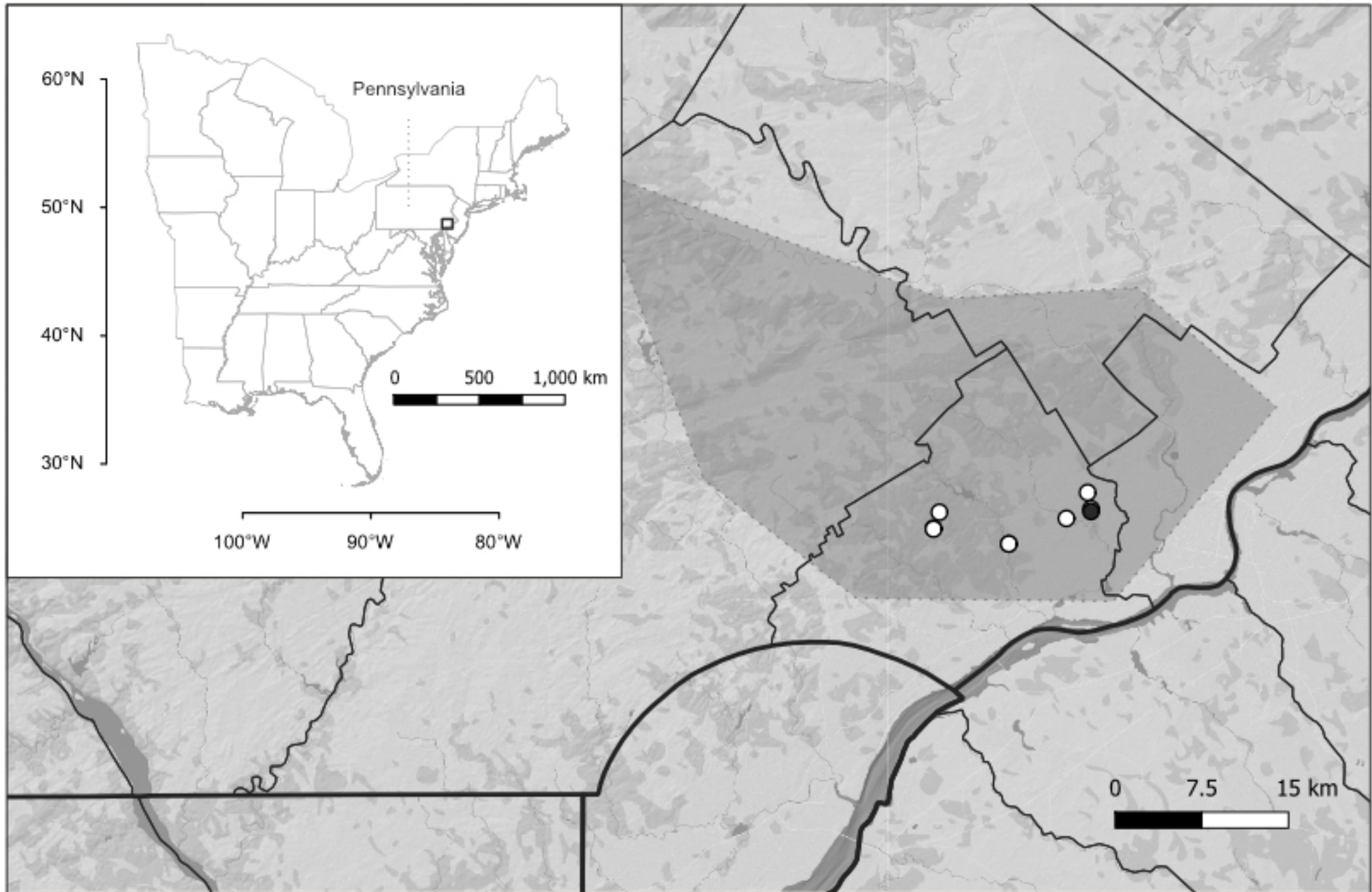792   https://www.fdacs.gov/content/download/10780/file/ent394.pdf
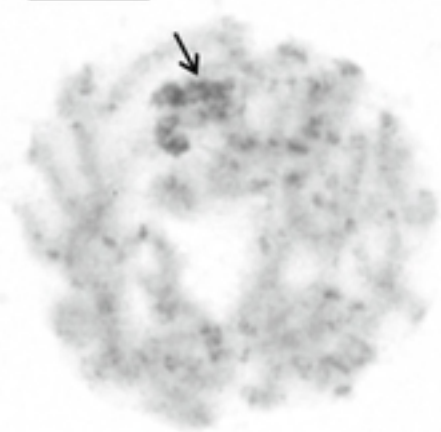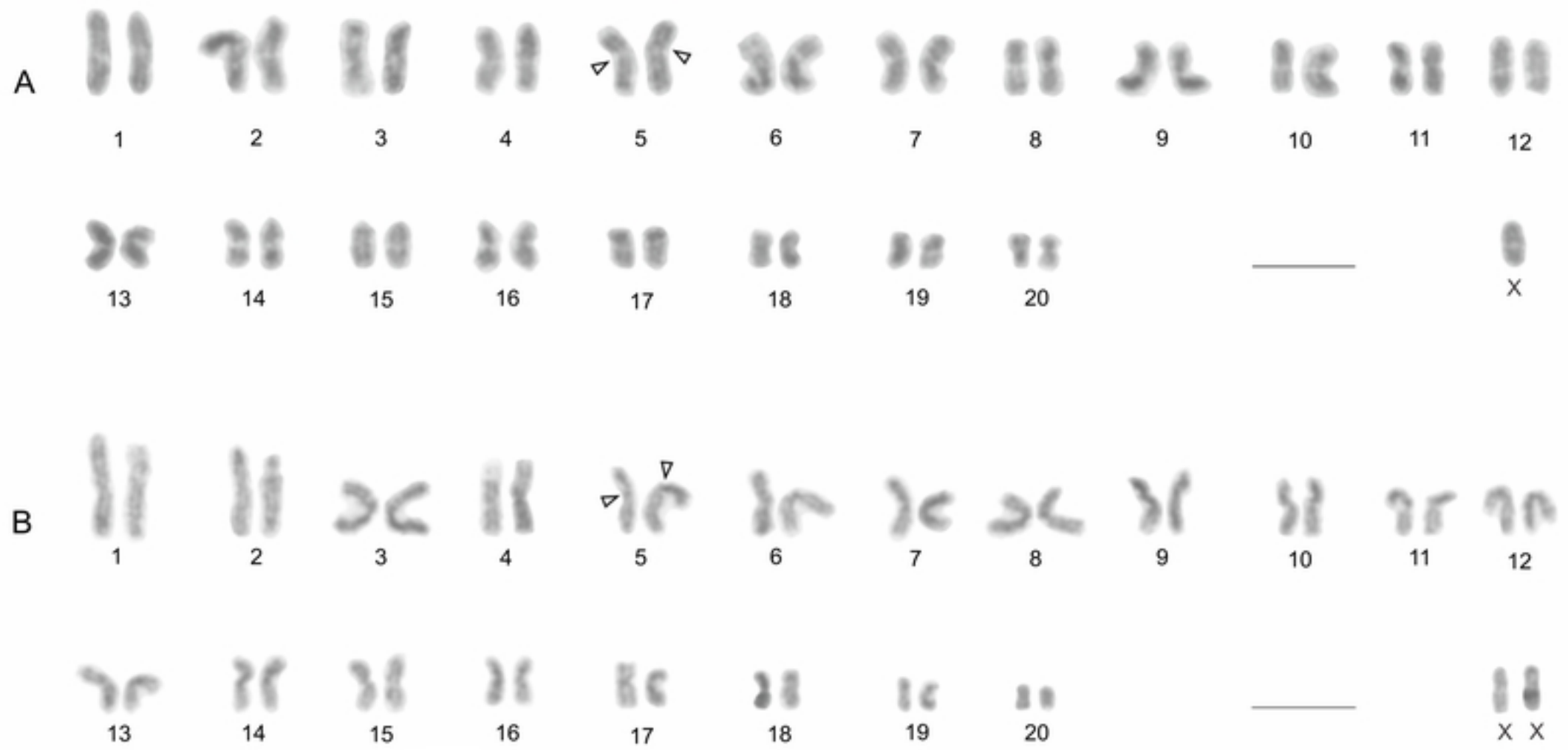
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Figure 6

Figure5

Figure4

Figure3

Figure2

Figure1