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1 **New karyotypic data for Asian rodents (Rodentia, Muridae)**

2 **with the first report of B-chromosomes in the genus *Mus***

3
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22
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24 **Running title:** Cytotaxonomy in Asian Rodents

25 **Abstract (216 words)**

26

27 Karyotypes of 18 rodent species collected in various localities in Thailand were analysed as
28 part of an epidemiological survey of the region using conventional cytogenetic techniques.
29 The aim was to re-assess the reliability of karyotype-based diagnoses of Thai rodents using an
30 updated taxonomic framework. Species examined include *Menetes berdmorei* (Sciuridae),
31 *Mus caroli*, *M. cervicolor* and *M. cookii*, *Hapalomys delacouri*, *Chiropodomys gliroides*, as
32 well as several representatives of most of the lineages of the Rattini tribe i.e. *Rattus exulans*,
33 *R. losea*, *R. tanezumi*, *Leopoldamys edwardsi*, *L. neilli*, *Maxomys surifer*, *Niviventer*
34 *fulvescens*, *Berylmys berdmorei*, *B. bowersi*, *Bandicota indica* and *B. savilei* (Muridae). The
35 first descriptions of G- and/or C-banding karyotypes are provided for several of these i.e., *B.*
36 *savilei*, *L. edwardsi*, *M. surifer*, *B. berdmorei*, *B. bowersi*, *N. fulvescens* and *H. delacouri*.
37 Although largely in agreement with available data, our findings on chromosomes morphology
38 differ slightly from those published for *L. edwardsi*, *M. surifer*, *B. savilei*, and the two
39 *Berylmys* species, *B. berdmorei*, and *B. bowersi*. In addition, we document the novel finding
40 of B-chromosomes in the genera *Berylmys*, *Bandicota* and the emblematic *Mus*. Importantly,
41 few species-specific chromosomal characters could be identified within most of the genera
42 investigated in our study and, contrary to previous claims, the usefulness of karyotypes for
43 diagnosing these Asian murid species appears limited.

44

45 **INTRODUCTION**

46

47 The non-ambiguous identification of species is critical for fundamental as well as applied
48 research, as it is the departure point for many aspects of comparative biology including
49 systematics (e.g., Sites & Marshall, 2003, and references therein), biodiversity assessments,
50 conservation (Crandall *et al.*, 2000; Moritz, 2000), biogeography (e.g., Avise 2009),
51 community ecology and pests control (e.g., Hoffman, 2002). In particular, the lack of accurate
52 species-specific identification of hosts is an important consideration for current
53 epidemiological surveys since 1) numerous pathogens are carried by a restricted range of
54 hosts, and 2) each rodent species possesses its own life traits. These two aspects are pivotal
55 since they may lead to important differences in the role a host species can play in circulation
56 and transmission of pathogens. For instance, a species may be an important reservoir for a
57 human pathogen while its close, and sometimes morphologically similar relatives, are not
58 (e.g., the two cryptic species *Mastomys natalensis* and *M. erythroleucus*, and their association
59 with the Lassa Fever arenavirus in Africa; Lecompte *et al.*, 2006). Additionally, some
60 species-specific ecological characteristics (such as commensality and hence the propensity to
61 transport with humans) and evolutionary properties (population structure and connectivity)
62 are also central to epidemiological processes (reviewed in Mills & Childs, 1998). In such a
63 context, karyotypic data has been shown to be valuable, especially for identifying rodents that
64 belong to sibling species complexes.

65

66 We carried out a survey of various rodent species found in different localities in Thailand as
67 part of an epidemiological survey in this region. In particular, we focused on murids (rats and
68 mice) that are suspected of being important reservoirs for several human pathogens (i.e., those
69 responsible for hantavirus-mediated hemorrhagic fevers, typhus, leptospirosis, among others).

70 The first goal was to develop identification “tools” for use at the species-level. That this
71 approach has application is illustrated by the Asian Hantaviruses (responsible for lethal
72 haemorrhagic fevers) / rodents association found in Cambodian *Rattus* specimens that could
73 not be discriminated at the species level (Reynes *et al.*, 2003).

74
75 Asia is the area of origin for the *Rattus sensu lato* complex (i.e., *Rattus sensu stricto* and
76 allied genera), a complex that includes the biological important index species, *R. norvegicus*
77 (Aplin *et al.*, 2003). Surprisingly, given their epidemiological importance, karyotypic data on
78 species in this complex are limited. Even more importantly, numerous systematic
79 modifications (reviewed in Wilson & Reeder, 2005) have been proposed since the pioneering
80 cytotaxonomic work published in the late 1960s to the 1970s (see Marshall, 1977, and
81 references therein), further complicating interpretations of correspondence between karyotype
82 and host species. For example, many (if not most) of the taxa that were originally considered
83 as different *R. rattus* subspecies (e.g., *tanezumi*, *argentiventer*, *rattus*) are now regarded as
84 synonyms or have been elevated to distinct species. In the same manner, many congeneric
85 species have been split into different genera (e.g., *Berylmys*, *Maxomys*, *Leopoldamys*,
86 *Niviventer*). Consequently, species-specific karyotypes are often difficult to delimit, thus
87 making a cytotaxonomic approach to species identification problematic.

88
89 In an attempt at redress, we performed a conventional cytogenetic analysis of 18 rodent
90 species: *Menetes berdmorei* (Sciuridae), *Mus caroli*, *M. cervicolor* and *M. cookii*,
91 *Chiropodomys gliroides*, *Hapalomys delacouri*, as well as representatives of most of the
92 lineages within the Rattini tribe (*sensu* Lecompte *et al.*, 2008), i.e. *Rattus exulans*, *R. losea*, *R.*
93 *tanezumi*, *Leopoldamys edwardsi*, *L. neilli*, *Maxomys surifer*, *Niviventer cf. langbianis*,

94 *Berylmys berdmorei*, *B. bowersi*, *Bandicota indica*, *B. savilei*. Our aim was to re-assess the
95 reliability of karyotype-based diagnoses of Thai rodents, in an updated taxonomic framework.

96

97 **MATERIAL and METHODS**

98

99 Thousands of rodents have been trapped and are currently under investigation as part of a
100 long-term epidemiological survey of rodent-borne pathogens in South Eastern Asia. Among
101 these, sixty five specimens were collected in 2006 and 2007 in Northern and North-Eastern
102 Thailand in the vicinity of the settlements Loei (17°29N 101°43E), Phrae (18°09N 100°08E)
103 and Kalasin (16°26N 103°31E) using locally made locally made cage-traps. Except where
104 otherwise detailed, taxonomy follows Carleton & Musser (2005). Species identifications were
105 based on external measurements, morphological features, and, in females, mammae formula
106 (Marshall, 1977; Corbet & Hill, 1992; Aplin *et al.*, 2003). Moreover, taking into account the
107 ambiguous status of some of these diagnostic characters, most of the murine specimens were
108 also investigated using molecular analyses (Tab. 1) involving several mitochondrial
109 (cytochrome b, CO1, and 16S rDNA) and nuclear (IRBP) gene fragments. The DNA-based
110 analyses will be presented elsewhere as a separate phylogenetic study (Pagès *et al.*, in prep.).
111 Importantly, however, and pivotal to the present work, our species-specific identifications
112 relied on correspondence in both morphological and molecular data.

113

114 The majority of chromosome preparations were done in the field using the air-drying method
115 on femoral bone marrow following yeast stimulation (Evans *et al.*, 1963; Lee & Elder, 1980).
116 In eleven instances (Tab. 1), intercostal tissue was sampled to establish fibroblast cell cultures
117 using conventional techniques. Freshly prepared hypotonic (0.75 M KCl) and fixative (acetic
118 acid-methanol 1:3) solutions were routinely used. Cell suspensions were stored in fixative and

119 transported to the laboratory where conventional staining, G- and C-banding (Seabright 1971;
120 Sumner 1972) and image capture using the Genus software (Applied Imaging), were
121 performed. A minimum of five complete metaphases were analysed for each specimen.
122 Diploid (2n), fundamental (NF; i.e., total numbers of chromosomal arms in one cell) and
123 autosomal fundamental (NFa; i.e., NF excluding sex chromosomes) numbers were
124 determined, and karyotypes were arranged according to chromosomes morphology and size
125 (conventional staining) and/or banding patterns. Chromosome nomenclature for *Rattus*
126 followed Yosida (1976, 1977a).

127

128 **RESULTS and DISCUSSION**

129

130 All specimens were analyzed using conventional staining, thus allowing an overall description
131 of the species-specific karyotypes (Tab. 1). In addition, G-banded karyotypes were
132 constructed where feasible. However, in several instances, slight variation and/or ambiguities
133 in NF/NFa values were detected through conventional staining (Giemsa only) reflecting the
134 difficulty in categorizing acrocentric/subtelocentric chromosomes where chromosomal
135 contraction can be a confounding factor. Although not uncommon in mammalian
136 cytogenetics, subtle differences in chromosome morphology (such as telocentric vs.
137 subtelocentric) has been considered important in murine cytotaxonomy where it has been used
138 to underpin the species identification of several taxa (e.g., Marshall, 1977, p. 407-408). In an
139 attempt to constrain ambiguities introduced by terminology, we have chosen to view all
140 chromosomes that have no visible short arm as telocentric and those that possess minute arms
141 as acrocentric elements (subtelocentric chromosomes in some schemes) for NF/NFa
142 assessment. If the short arms were more pronounced and clearly and repeatedly observed,
143 chromosomes were considered to be as bi-armed (submetacentric and metacentric).

144

145 *Menetes berdmorei* (Blyth, 1849) (Sciuridae)

146

147 The only squirrel (which is a sciurid, not a murid rodent) investigated in this study had a
148 $2n=38$ chromosome number. Most of the chromosomes were metacentric or submetacentric
149 except for the five smallest pairs that were either telocentric or acrocentric (NF=76, NFa=72).
150 This is in agreement with data previously published by Viegas-Péquignot *et al.* (1986) and
151 Richard *et al.* (2003) on this species, and also specimens analysed by Marshall (1977) from
152 Thailand. As a result the karyotype is not repeated here.

153

154 *Rattus exulans* (Peale, 1848), *R. losea* (Swinhoe, 1871) and *R. tanezumi* (Temmink, 1844)

155

156 In total 26 specimens of *Rattus sensu stricto* were karyotyped (Tab. 1; data not shown). These
157 included *R. losea* (n=5), *R. tanezumi* (n=13) and *R. exulans* (n=8). All but one specimen were
158 determined to have a diploid number of 42 with NF values ranging from 56-61, 58-60 and 54-
159 59 in *R. exulans*, *R. losea* and *R. tanezumi*, respectively. The $2n=43$ *R. tanezumi* specimen
160 was characterized by the presence of a G-negative and C-positive B-chromosome (data not
161 shown), as has previously been documented in this taxon (Yosida, 1976, 1977b; Gadi &
162 Sharma, 1983). The largely overlapping NF values are attributable to variation in the short
163 arms (p arms) of several pairs resulting in their classification as acrocentric and/or
164 submetacentric homologs (see Table 2). The polymorphisms most likely correspond to
165 variation in pairs 1, 9 and 13 of *Rattus r. tanezumi* (now named *R. tanezumi*; see Wilson &
166 Reeder, 2005) *sensu* Yosida and colleagues (1971a, 1971b, 1976 and 1977a). This variation in
167 centromere position has been noted in several *Rattus* species and subspecies (Yosida *et al.*,
168 1971a, 1971b, 1974; Yosida, 1973, 1977a; Yosida & Sagai, 1973, 1975; Gadi & Sharma,

169 1983; Motokawa *et al.*, 2001) and is interpreted to result from pericentric inversion (Yosida *et*
170 *al.*, 1971a, 1971b; Yosida, 1976, 1977a) and/or heterochromatin amplification/deletion
171 (Yosida & Sagai, 1975; Baskevich & Kuznetsov, 1998). However, the chromosomes and/or
172 the segments involved are small and thus difficult to interpret, even when using G-banding,
173 and the mechanism(s) underpinning this variation remains moot.

174
175 Importantly, Marshall (1977) proposed a number of apparently fixed (diagnostic) features
176 among *R. exulans*, *R. losea* and *R. tanezumi* (which is synonymous with *R. r. thai sensu*
177 Marshall; see Wilson & Reeder, 2005) in Thailand. On the basis of conventional staining,
178 Marshall argues (pp. 407-408) that: i) pair 1 is acrocentric in *R. tanezumi* and submetacentric
179 in *R. exulans* and *R. losea*, ii) pair 9 is submetacentric in *R. losea* but acrocentric in *R. exulans*
180 and *R. tanezumi*, and iii) pair 11 (most probably corresponding to pair 13 *sensu* Yosida *et al.*)
181 is submetacentric in *R. exulans*, whereas it is acrocentric in *R. losea* and *R. tanezumi*.
182 However, taking other studies into account (see above) and our own data (Table 2), it appears
183 that none of these chromosomal morphs (1, 9 or 13) can be considered as species-specific.
184 Moreover, heterozygotes for each of these three pairs were found in *R. exulans* and *R.*
185 *tanezumi* (our study, Tab. 2), suggesting that these chromosomal polymorphisms were present
186 in their last common ancestor and are therefore ancestral to the *Rattus sensu stricto* clade
187 (Yosida *et al.*, 1971a, 1971b; Yosida & Sagai, 1975, Yosida, 1977) and date back ~2.2 My
188 (Robins *et al.*, 2008).

189
190 *Leopoldamys edwardsi* (Thomas, 1882) and *L. neilli* (Marshall Jr, 1976)

191
192 The five *L. edwardsi* specimens investigated in this study all display identical karyotypes
193 (Fig. 1) with 2n=42 chromosomes. Most of the autosomes are acrocentric, except for four of

194 the five largest autosomal pairs (pairs 1, 2, 3 and 5 in Fig. 1) which possess short arms, as
195 well as the three smallest ones which are metacentric (pairs 18, 19 and 20). The X is a
196 medium size acrocentric chromosome, whereas the Y is C-positive and dot-like (and most
197 likely acrocentric) element (NFa=54). Clearly observable blocks of C-positive
198 heterochromatin were detected in several pericentromeric and telomeric regions, as well as in
199 an intercalary position on one of the largest pairs (Fig. 1b; pair 2 on Fig. 1a).

200

201 Although *L. edwardsi* (from an unknown locality) was recently proposed to have $2n=44$
202 chromosomes (Sablina *et al.*, unpubl., quoted in the “Atlas of Mammalian Chromosomes”, ed.
203 S.J. O’Brien *et al.*, 2007), our karyotypic results are in agreement with other reports (Yong,
204 1970; Rickart & Musser, 1993, and references therein). However, there is also a difference in
205 the morphology of the Y chromosomes: the Y identified by Yong (1970) is a normal sized
206 acrocentric, while our Y clearly appears as a dot like element (Fig 1b), suggesting there may
207 be more fundamental differences underlying these observations.

208

209 A single *Leopoldamys* female trapped in the vicinity of limestone karsts was referred to as *L.*
210 *neilli* on the basis of morphology as well as unambiguous molecular results (Pagès *et al.*, in
211 prep.). Even though our preparation was of poor quality (data not shown), making G-banding
212 analysis problematic, the specimen unambiguously displayed $2n=44$ chromosomes, with two
213 large submetacentric and two small metacentric pairs (NF=52). These findings are in good
214 agreement with previous reports (NF=52 in Marshall, 1977) but differ from the NF=50 noted
215 by Rickart & Musser (1983).

216

217 It would appear, therefore, that the two *Leopoldamys* species can be distinguished by
218 karyotypes: *L. edwardsi* with $2n=42/NF=56$ and *L. neilli* with $2n=44/NF=52$. A third species,

219 *L. sabanus*, which is morphologically similar to *L. edwardsi*, has also been described from
220 Thailand (Wilson & Reeder, 2005). The *L. sabanus* karyotype differs slightly from that of *L.*
221 *edwardsi* since Marshall (1977) recorded a $2n=42/NF=54$ complement for this species.
222 Unfortunately, no material was available to us to address the delimitation of the *L. edwardsi* /
223 *L. sabanus* species. However, we anticipate that karyotypic differences between these two
224 taxa are subtle, therefore unreliable for diagnostic purposes.

225

226 *Niviventer fulvescens*

227

228 Taxonomy within *Niviventer* is in need of revision (Wilson & Reeder, 2005). The results of
229 our preliminary molecular (Pagès *et al.*, in prep.) and morphological (Chaval, unpubl.)
230 investigations are concordant and show that the three specimens analysed karyotypically by
231 us form a natural grouping and are referred to herein as *N. fulvescens* (Pagès *et al.*, in prep.).
232 Of these three specimens, a male was used to establish a fibroblast culture but the resulting
233 lines appear to have undergone *de novo* rearrangement in culture. Nonetheless, the rearranged
234 karyotype corresponds relatively well to the bone marrow karyotypes of the other two
235 specimens which were both characterized by $2n=46$ (NFa=52; Fig. 2). All chromosomes are
236 acrocentric except for the submetacentric pair 2, as well as the three of the shortest autosomal
237 pairs which are metacentric. The X is a large acrocentric chromosome, and the Y is a tiny
238 acrocentric element which is entirely C-positive (data not shown). As far as we know, the G-
239 banding karyotype of *N. fulvescens* (Fig. 2) is presented here for the first time.

240

241 There are few published karyotype reports on subspecies/species of *Niviventer* and these are
242 limited to using conventional staining, the only exception being Wang *et al.* (2003) on *N.*
243 *confucianus*. Except for tiny differences in centromeric placement, *N. rapit*, *N. bukit*, *N.*

244 *hinpoon* (Yong, 1969; Markvong *et al.*, 1973; Marshall, 1977), *N. langbianis* (Baskevich &
245 Kuznetsov, 2000), *N. fulvescens* (Duncan *et al.*, 1970, 1974) and *N. cremoriventer* (Yong,
246 1969) are all invariant and closely resemble the cytotype that we present here for *N.*
247 *fulvescens*. It is important to note that *N. rapit* and *N. bukit* from Thailand have since then
248 been collapsed into *N. fulvescens* (see Musser, 1981; Abe, 1983). The cytogenetic criteria
249 proposed to discriminate all these *Niviventer* species are based on subtle shape differences of
250 some pairs (see Table 2 in Wang *et al.*, 2003) which are most probably due to variation in
251 heterochromatin, as observed between *N. tenaster*, *N. fulvescens* and *N. langbianis* (all from
252 Vietnam) following C-banding protocols (Baskevich & Kuznetsov, 2000). Although we
253 cannot state with certainty, it is highly probable that karyotypic variations within the genus
254 will be too obscure to provide reliable cytotaxonomic identification.

255

256 *Maxomys surifer* (Miller, 1900)

257

258 Five males and one female of *M. surifer* were analysed and the G-banded karyotype is
259 presented for the first time (Fig. 3). All specimens displayed $2n=52$, with seven metacentric
260 (pairs 4, 5 and 14 in Fig. 3) or submetacentric (pairs 10, 12, 18 and 24) chromosomal pairs
261 (NF=66). The Y chromosome is the smallest element, is entirely heterochromatic (Fig. 3b),
262 and appears acrocentric in morphology. This is in agreement with available data for *M. surifer*
263 from South East Asia (see Rickart & Musser, 1993, and references therein) including
264 Thailand (Marshall, 1977). However, some differences exist between these studies and the
265 results of the present report especially with respect to the identification of the X chromosome.
266 The X was described as a metacentric in specimens collected from localities in Thailand
267 (Marshall, 1977), Vietnam and Malaysia (see Rickart & Musser, 1993, and references
268 therein), resulting in $2n=52/NFa=62$. In sharp contrast, the X chromosome is one of the

269 largest chromosomal elements in specimens analysed by us, and is clearly acrocentric. Zoo-
270 FISH analysis using a *R. norvegicus* X painting probe (Badenhorst *et al.*, unpubl.) allowed us
271 to confirm this result. Our *M. surifer* specimens are consequently characterized by NFa=64,
272 and not NFa=62. When one considers our findings on the morphology of the X chromosome,
273 the difference in NFa (NFa=62 vs 64) may be due to (i) a misidentification of the X in the
274 past, (ii) a polymorphism that would involve the X chromosome, or (iii) the co-existence of
275 different but cryptic *Maxomys* species that differ in the morphology of the X chromosome.
276 Further sampling and karyotyping will be required to address this point.

277

278 *Berymys berdmorei* (Anderson, 1879) and *B. bowersi* (Blyth, 1851)

279

280 A single *B. berdmorei* female, as well as one male and one female of *B. bowersi* were
281 karyotyped. All three specimens had $2n=40$. The karyotype comprised two large (pairs 1 and
282 2 in Fig. 4 and 5), four medium-size (pairs 5, 6, 9 and 11) and one small submetacentric pairs
283 (pair 18), one large (pair 3), five medium-size (pairs 12-16) and one small metacentric pair
284 (pair 17), and three medium-size (pairs 4, 7, 8 and 10) and one short acrocentric (pair 19)
285 pairs of chromosomes (NFa=64; see Fig. 4 and 5). The X is a medium-size acrocentric
286 chromosome and the *B. bowersi* Y is a small acrocentric element. Our results are in good
287 agreement with previous reports (reviewed in Marshall and Newcomb, 1983) that suggested
288 $2n=40$ and NFa=64 for both species. An additional female *B. berdmorei* specimen was found
289 to have $2n=41$ due to the presence of an extra C-negative metacentric element that did not
290 match with any of the other elements of the karyotype using G-banding comparison (Fig. 4)
291 suggesting that this may be a B-chromosome. To our knowledge, this is the first report of B-
292 chromosomes in this genus.

293

294 Our results show some differences to those of Yong (1968) and Yosida (1973) who identified
295 six pairs of acrocentric, eight pairs of metacentric and five pairs of subtelocentric autosomes;
296 they also show a medium sized acrocentric X chromosome and a small acrocentric Y in
297 specimens of *B. bowersi*. This mirrors other conflicting reports regarding the *B. berdmorei*
298 karyotype. Marshall (1977) described six telocentric, six subtelocentric and seven metacentric
299 pairs of autosomes in *B. bowersi* from Thailand, whereas Yong (1968) recorded eight pairs of
300 metacentrics, nine pairs of subtelocentrics, two pairs of acrocentric autosomes and a medium
301 sized subtelocentric X in specimens from Malaysia. Conversely, Markvong *et al.* (1973) and
302 Duncan *et al.* (1970) identified five pairs of subtelocentrics, eight pairs of metacentrics and
303 six pairs of acrocentrics in this species in Thailand and Vietnam. The X and Y chromosomes
304 of *B. bowersi* described by Yong (1968) and Yosida (1973) are comparable to our own.
305 Conversely the *B. berdmorei* X has previously been reported as subtelocentric (Yong, 1968),
306 while our specimens clearly possessed an acrocentric X, in agreement with both Duncan *et al.*
307 (1970) and Markvong *et al.* (1973). Furthermore, we were unable to retrieve the two species-
308 specific differences that were proposed to differentiate the karyotypes, i.e. a subtelocentric vs.
309 acrocentric pair 6, and an acrocentric vs. subtelocentric pair 11 in *B. berdmorei* and *B.*
310 *bowersi*, respectively (see Marshall, 1977). Given that our C-banding data show the presence
311 of several C-positive short arms (Fig. 4 and 5) which may be responsible for variations in
312 chromosome morphology (i.e., the interpretation of acrocentric vs. subtelocentric), it seems
313 prudent to conclude that the two *Berylmys* species cannot be distinguished cytogenetically
314 with any confidence.

315

316 *Bandicota indica* (Bechtein, 1800) and *B. savilei* Thomas, 1916

317

318 Eight bandicoot-rats, including two *B. indica* (one male, one female) and six *B. savilei* (four
319 males, two females) specimens were karyotyped.

320

321 The *B. indica* specimens were characterized by $2n=44$ and 45 , respectively (data not shown).

322 Unfortunately, well resolved G- and C-banded chromosomes could not be produced due to the

323 poor quality of our preparations. That said, the $2n=45$ male clearly possessed 26 bi-armed

324 chromosomes (NF=71) including a moderately large submetacentric X. The rest of the

325 karyotype was composed of acrocentric elements, most probably including the Y (which

326 would result in NFa=68) although its unambiguous identification was not possible from our

327 preparations. The $2n=44$ female was characterized by the same karyotypic morphology,

328 except that it possessed one more acrocentric but one less bi-armed chromosome (data not

329 shown). Although the difference between the $2n=44$ and $2n=45$ karyotypes may suggest a

330 Robertsonian polymorphism in *B. indica*, based on our data we are unable to reach a

331 definitive conclusion. Consequently, other explanations (e.g., X-autosome translocations, B

332 chromosomes) cannot be excluded at this stage. For example, *B. indica* has been previously

333 reported to contain 1-3 B chromosomes (Gadi *et al.*, 1982; Gadi & Sharma, 1983).

334 Importantly, however, our results are slightly different to those previously published for this

335 species collected from Thailand ($2n=46$; Markvong *et al.*, 1973; Marshall, 1977), but are

336 consistent with data on specimens from India ($2n=42$ for *B. indica indica*, and $2n=44$ for *B.*

337 *indica nemorivaga*; Ranjini, 1967; Avirachan *et al.*, 1971; Gadi and Raman, 1977; Gadi &

338 Sharma, 1983). When taken together, the variation in $2n$ suggests a more complex systematic

339 situation than currently thought within *B. indica*, and/or that there is a chromosomal

340 polymorphism that remains to be described.

341

342 Three males and two females of *B. savilei* were characterised by $2n=43$ (Fig. 6). The diploid
343 number of an additional male specimen was determined to be $2n=45$. Unfortunately, as for *B.*
344 *indica*, our preparations were of very poor quality thus greatly limiting the description of
345 these karyotypes and our interpretation of their differences. The fundamental number could
346 not be determined confidently from these five specimens. Nevertheless, we were able to
347 establish a cell line for one female allowing for a more detailed investigation of the *B. savilei*
348 karyotype. This specimen had $2n=43$ comprising 23 meta- and submetacentric elements
349 (NF=64; Fig. 6). Among the bi-armed chromosomes two correspond to the large metacentric
350 X pair (as confirmed by Zoo-FISH using a *Rattus norvegicus* X paint; Badenhorst *et al.*,
351 unpubl.), and one was a B chromosome (Fig. 6a). C-banding showed that the X long arms as
352 well as several acrocentric and metacentric centromeric regions were C-positive (Fig. 6b).
353 More surprisingly, the B-chromosome did not appear strongly stained following C-band (Fig.
354 6b). We are not aware of previous reports of B-chromosomes in *B. savilei*.

355
356 The karyotype described above differs from previous reports for specimens of this species
357 collected in Thailand ($2n=44$, NFa=58; Markvong *et al.*, 1973). Markvong *et al.*, (1973)
358 described a karyotype consisting of six pairs of small metacentrics and two pairs of
359 subtelocentrics and thirteen pairs of acrocentrics. Also, the largest autosomal pair was clearly
360 submetacentric. Furthermore, the X of *B. savilei* was previously identified as a medium-sized
361 acrocentric which differentiates it from other lesser bandicoot rats (Markvong *et al.*, 1973;
362 Gadi & Sharma, 1983). In absence of comparable data, we are unable to explain such
363 differences. However, in our specimen the X was identified unambiguously by Zoo-FISH
364 analysis (Badenhorst *et al.*, unpubl.) as a large submetacentric chromosome. Therefore, males
365 of *B. savilei* should have a $2n=43+1B$ karyotype (i.e., XY1Y2 males). Clearly, this requires
366 confirmation by further cytogenetic investigations.

367

368 *Hapalomys delacouri* Thomas, 1927

369

370 A fibroblast cell culture was established from a male *H. delacouri* specimen caught in the
371 vicinity of Loei, and our data are the first for this species from Thailand. Its karyotype was
372 characterized by $2n=48$ chromosomes and an $NFa=92$ (Fig. 7). All the autosomes were bi-
373 armed (metacentric or submetacentric). The metacentric X and the acrocentric Y were easily
374 recognizable since they were respectively the largest and the smallest elements in the
375 karyotype. Our findings differ significantly from published data on *H. longicaudatus* (Yong *et*
376 *al.*, 1982) in that these authors describe specimens from Malaysia as having $2n=50$, with the
377 X and Y chromosomes having similar morphology; all the autosomes were reported as
378 acrocentric. This suggests that cytotaxonomy may be a valuable diagnostic tool within the
379 genus. Our results constitute the first karyotypic data for this very poorly documented species.
380 Interestingly, two classes of C-positive material were identified on staining intensity
381 suggesting a different repeat composition for this heterochromatic material (Fig. 7b). The
382 genome appears to be particularly rich in heterochromatin, with some chromosome pairs
383 displaying entirely heterochromatic long arms.

384

385 *Chiropodomys gliroides* (Blyth, 1856)

386

387 A fibroblast cell culture was established from a male *C. gliroides* specimen that possessed an
388 entirely acrocentric chromosomal complement ($2n=42$, $NF=42$), including the X and Y
389 chromosomes ($NFa=40$). Unfortunately, the preparations were of poor quality and
390 construction of a G-banded karyotype was not possible. Nevertheless, strongly positive C-
391 bands could be clearly observed in all pericentromeric regions, whereas the Y chromosome

392 was entirely heterochromatic, although less heteropycnotic than the pericentromeric material
393 of the autosomes (Fig. 8). These results are in partial agreement with previous data
394 concerning this very poorly documented species. Yong (1973, 1983) reported that eight
395 Malayan specimens were characterized by $2n=42$ with most chromosomes being acrocentric
396 in morphology with C-positive pericentromeric regions (Yong, 1983). Additionally there was
397 one metacentric chromosome pair, a submetacentric chromosome pair, and the sex
398 chromosomes were bi-armed. These bi-armed elements were all clearly absent in our single
399 specimen of this species (Fig. 8). At this point, we cannot distinguish whether the differences
400 are intraspecific (i.e. represent polymorphisms), or that they underpin the recognition of
401 apparently cryptic species within *C. gliroides*.

402

403 *Mus caroli* (Bonhote, 1902), *M. cervicolor* Hodgson, 1845 and *M. cookii* Ryley, 1914

404

405 Seven male and seven female mice, trapped close to or within pineapple fields in Loei, were
406 characterized by an identical $2n=40$ acrocentric karyotype (NFa=38). The karyotypes of the
407 Thailand species *M. musculus castaneus*, *M. caroli*, *M. cervicolor*, *M. cookii*, *M. shorridgei*
408 and *M. fragilicauda* (see Auffray *et al.*, 2003, and references therein) all have the same
409 invariant karyotype (Auffray *et al.*, 2003) indistinguishable from that evidenced by our
410 specimens. Molecular typing using rDNA16S and rDNA28S nucleotide sequences of the 14
411 specimens analysed by us allowed the identification of three different species in our sample,
412 *M. caroli*, *M. cervicolor* and *M. cookii* (A. Orth, pers. comm.). Several of these animals were
413 caught in the same trap lines, often only meters apart, thus representing another report of true
414 syntopy as has been similarly suggested for *M. caroli*, *M. cervicolor* and *M. fragilicauda* in
415 Thailand (Auffray *et al.*, 2003).

416

417 In addition, two female specimens, identified as *M. cookii* on nucleotide sequences (A. Orth,
418 pers. comm.), had $2n=41$ chromosomes (Fig. 8). The two karyotypes each comprised 40
419 acrocentric chromosomes, in addition to one unpaired medium-size metacentric and one
420 unpaired acrocentric element respectively. These two unpaired chromosomes are G-negative
421 (not shown) and C-positive (Fig. 8b) and are considered by us to be B-chromosomes. To our
422 knowledge, this is the first report of B-chromosomes in the genus *Mus*. Although several
423 hypotheses have been proposed to explain some aspects of the biology of these extra, non-
424 Mendelian chromosomes (reviewed in Camacho *et al.*, 2000), little is known about their
425 origin and their impact on the genomes, especially in mammals. Keeping in mind the
426 importance of the mouse as a biological model, this observation clearly merits further
427 investigation.

428

429 **Conclusion: chromosomal evolution in Thai rodents and epidemiological implications**

430

431 Despite a central role as reservoirs and/or vectors of several human diseases, reliable
432 systematic data necessary to underpin accurate species-specific geographical, ecological and
433 genetic information on Asian rodents are limited, and many taxonomic associations are
434 unclear. Cytotaxonomy has been of great help in similar epidemiological investigations on
435 other continents i.e., Africa), frequently providing unambiguous identification of species
436 including those that are morphologically cryptic (e.g., Duplantier *et al.*, 1990; Volobouev *et*
437 *al.*, 2002; Dobigny *et al.*, 2003; Granjon & Dobigny, 2003; Rambau *et al.*, 2003; Veyrunes *et*
438 *al.*, 2004). However, apart from *Hapalomys*, the preliminary results presented here suggest
439 that this does not hold for the Asian species surveyed in our study. Although most genera can
440 routinely be discriminated on karyotype (as they often can on morphology), few species-
441 specific chromosomal characters could be identified within genera investigated herein. For

442 instance, although *L. neilli* and *L. edwardsi* could be delimited cytogenetically, the two
443 *Berymys*, three *Mus*, three *Rattus* species analysed by us are indistinguishable on karyotype.
444 Although it remains to be confirmed, a similar situation may exist within *Niviventer*, as well
445 as between *L. edwardsi* and *L. sabanus*. Karyotypic criteria that have previously been claimed
446 to be diagnostic appear in our analyses to simply represent ancestral polymorphisms that are
447 still undergoing idiosyncratic lineage sorting (i.e., hemiplasic characters *sensu* Avise &
448 Robinson, 2008; Robinson *et al.*, 2008), thus impacting on the usefulness of these as
449 diagnostic markers in species identification. Our findings suggest that new diagnostic features
450 should be sought as a matter of urgency for use in these epidemiologically important rodent
451 groups. DNA-based methods such as primer-specific analysis that have been used
452 successfully in Africa (e.g., Lecompte *et al.*, 2005) and Europe (e.g., Michaux *et al.*, 2001)
453 are compelling candidates that should be prioritised for testing in Asian murids.

454

455

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- 603
- 604

605 **Table and Figure Legends**

606

607 Table 1: Specimens investigated in the present study. Those that were diagnosed using DNA
608 sequencing are noted as “+” in the Id. DNA column. Individual numbers refer to the CBGP
609 Asian rodent collection (resp. Y. Chaval, Montpellier, France). Sex, origin, diploid (2n) and
610 autosomal fundamental (NFa) numbers, type of biological material (bm: bone marrow; cc:
611 cell culture), sex chromosomes morphology (X/Y) as well as number of B-chromosomes (Bs)
612 are provided for each animal.

613

614 Table 2: Chromosomal polymorphism observed for pairs 1, 9 and 13 (*sensu* Yosida and
615 colleagues; see text for details) in the *Rattus* specimens investigated here. M and A
616 correspond to meta-/submetacentric and acrocentric morphs, respectively. Like for Table 1,
617 individual numbers refer to the CBGP Asian rodent collection (resp. Y. Chaval, Montpellier,
618 France).

619

620 Fig. 1: G-banded karyotype (a) and C-banded metaphase (b) of a male *Leopolodamys*
621 *edwardsi* (n°R5239). Arrows indicate C-positive intercalary bands and minute Y.

622

623 Fig. 2: G-banded karyotype (a) and C-banded metaphase (b) of a female *Niviventer fulvescens*
624 (n°R4519).

625

626 Fig. 3: G-banded karyotype (a) and C-banded metaphase (b) of a male *Maxomys surifer*
627 (n°R4404). The arrow indicates the C-positive Y chromosome.

628

629 Fig. 4: G-banded karyotype (a) and C-banded metaphase (b) of a female *Berylmys berdmorei*
630 (n°R4406).

631

632 Fig. 5: G-banded karyotype (a) and C-banded metaphase (b) of a male *Berylmys bowersi*
633 (n°R4400). The arrow indicates the C-positive Y chromosome.

634

635 Fig. 6: G-banded karyotype (a) and C-banded metaphase (b) of a female *Bandicota savilei*
636 (n°R4408). Arrows indicate the X chromosomes.

637

638 Fig. 7: G-banded karyotype (a) and C-banded metaphase (b) of a male *Hapalomys delacouri*
639 (n°R5237). The arrow indicates the C-negative Y chromosome.

640

641 Fig. 8: C-banded metaphase of a male *Chiropodomys gliroides* (n°R5238).

642

643 Fig. 9: Conventionally stained (a) and C-banded (b) metaphases of one female *Mus cooki*
644 (n°R4105) showing the strongly C-positive and metacentric B-chromosome (arrows).

645

646 Table 1

647

Species	Id. DNA	Individuals			material	Cytogenetics			
		individual	sex	origin		2N	X/Y	NFa	Bs
<i>Menetes berdmorei</i>	-	R4002	F	Kalasin	bm	38		72	
<i>Rattus losea</i>	+	R4203	F	Phrae	bm	42	A	58	
	+	R4229	M	Loei	bm	42	A/A	58	
	+	R4230	M	Loei	bm	42	A/A	57	
	+	R4260	F	Loei	bm	42	A	58	
	+	R4724	F	Loei	cc	42	A	56	
<i>Rattus tanezumi</i>	+	R4113	M	Loei	bm	42	A/A	54	
	+	R4481	F	Phrae	bm	42	A	55	
	+	R4003	F	Kalasin	bm	42	A	57	
	-	R4008	M	Kalasin	bm	42	A/A	55	
	+	R4016	F	Phrae	bm	42	A	52	
	-	R4096	F	Loei	bm	42	A	52	
	-	R4145	F	Phrae	bm	42	A	54	
	+	R4182	F	Phrae	bm	42	A	56	
	+	R4436	M	Phrae	bm	43	A/A	59 ?	1
	-	R4447	M	Phrae	bm	42	A/A	55	
	-	R4450	M	Phrae	bm	42	A/A	56	
	-	R4542	F	Loei	bm	42	A	56	
	+	R4075	M	Loei	bm	42	A/A	57	
<i>Rattus exulans</i>	-	R4033	F	Phrae	bm	42	A	58	
	-	R4035	F	Phrae	bm	42	A	59	
	-	R4100	F	Loei	bm	42	A	59	
	+	R4103	M	Loei	bm	42	A/A	58	
	-	R4104	M	Loei	bm	42	?	?	
	-	R4112	M	Loei	bm	42	A/A	54	
	+	R4140	F	Phrae	bm	42	A	?	
	-	R4216	M	Loei	bm	42	A/A	58	
<i>Bandicota indica</i>	+	R4000	M	Kalasin	bm	45	??	?	
	+	R4265	F	Loei	bm	44	?	?	
<i>Bandicota savilei</i>	+	R4141	M	Phrae	bm	43	??	?	
	+	R4142	M	Phrae	bm	43	??	?	
	+	R4143	M	Phrae	bm	45	??	?	
	+	R4017	F	Phrae	bm	43	?	?	
	+	R4408	F	Loei	cc	43	SM	60	1
	+	R4021	M	Phrae	bm	43	??	?	
<i>Leopoldamys edwardsi</i>	+	R5239	M	Loei	cc	42	A/A	54	
	+	R5240	M	Loei	cc	42	A/A	54	
	+	R4070	M	Loei	bm	42	A/A	54	
	+	R4098	M	Loei	bm	42	A/A	54	

	+	R4222	F	Loei	bm	42	A	54		
<i>Leopoldamys neilli</i>	+	R4477	F	Phrae	bm	44	A?	50		
<i>Maxomys surifer</i>	+	R4099	M	Loei	bm	52	A/A	64		
	+	R4107	M	Loei	bm	52	A/A	64		
	+	R4108	M	Loei	bm	52	A/A	64		
	+	R4223	M	Loei	bm	52	A/A	64		
	+	R4259	F	Loei	bm	52	A	64		
	+	R4404	M	Loei	cc	52	A/A	64		
	<i>Niviventer fulvescens</i>	+	R4071	M	Loei	bm	46	A/A	52	
+		R4409	M	Loei	cc	44 (rearr.)				
+		R4519	F	Loei	bm	46	A	52		
<i>Berylmys berdmorei</i>	+	R4266	F	Loei	bm	40	A	62		
	+	R4406	F	Loei	cc	41	A	64	1	
<i>Berylmys bowersi</i>	+	R4102	F	Loei	bm	40	A	62		
	+	R4400	M	Loei	cc	40	A/A	62		
<i>Hapalomys delacouri</i>	+	R5237	M	Loei	cc	48	M/A	92		
<i>Chiropodomys gliroides</i>	+	R5238	M	Loei	cc	42	A/A	40		
<i>Mus cooki</i>	+	R4074	M	Loei	bm	40	A/A	38		
	+	R4105	F	Loei	bm	41	A	40	1	
	+	R4215	M	Loei	bm	40	A/A	38		
	+	R4097	M	Loei	bm	40	A/A	38		
	+	R4101	F	Loei	bm	40	A	38		
	+	R4106	M	Loei	bm	40	A/A	38		
	+	R4110	M	Loei	bm	40	A/A	38		
	+	R4111	F	Loei	bm	40	A	38		
	+	R4146	F	Phrae	bm	40	A	38		
	+	R4264	F	Loei	bm	40	A	38		
	+	R4147	F	Phrae	bm	41	A	39	1	
	<i>Mus cervicolor</i>	+	R4109	M	Loei	bm	40	A/A	38	
		+	R4183	M	Phrae	bm	40	A/A	38	
<i>Mus caroli</i>	+	R4240	F	Loei	bm	40	A	38		

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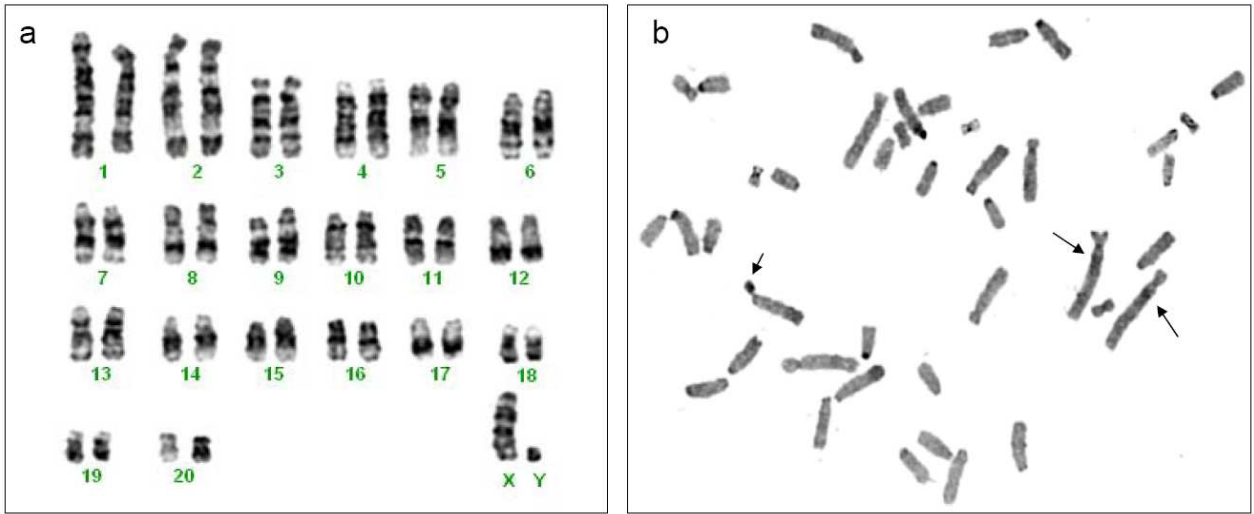
651 Table 2

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Species	Individuals	Polymorphism in <i>Rattus</i> species		
		pair 1	pair 9	pair 13
<i>Rattus losea</i>	R4203	MM	MM	AA
	R4229	MM	MM	AA
	R4230	MM	MM	AA
	R4260	MM	MM	AA
	R4724	MM	AA	AA
<i>Rattus tanezumi</i>	R4113	AA	AA	AA
	R4481	AA	AM	AA
	R4003	AM	AM	AM
	R4008	AA	AM	AA
	R4016	AA	AA	AA
	R4096	AA	AA	AA
	R4145	AA	AA	AA
	R4182	AA	MM	AA
	R4436	AA	?	?
	R4447	AM	AA	AA
	R4450	AM	AM	AA
	R4542	AA	MM	AA
	R4075	AM	MM	AA
<i>Rattus exulans</i>	R4033	MM	MM	AA
	R4035	MM	MM	AA
	R4100	MM	MM	AM
	R4103	MM	MM	AA
	R4104	?	?	?
	R4112	MM	AA	AA
	R4140	?	?	?
	R4216	MM	AA	MM

653 Fig. 1

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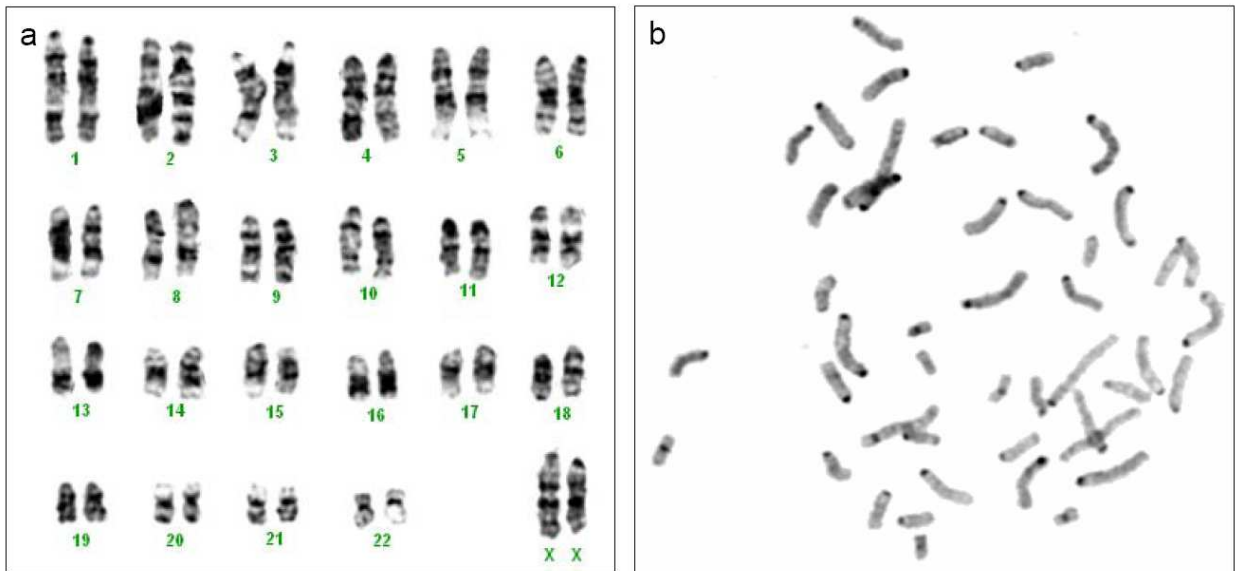


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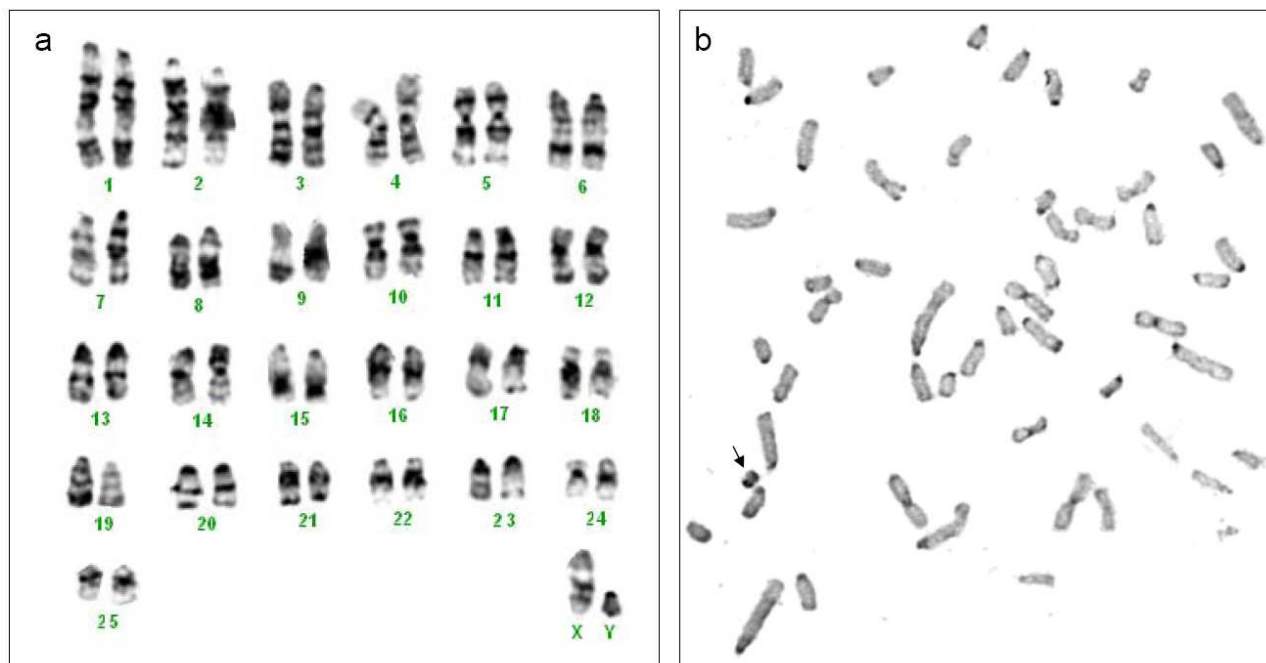
658 Fig. 2



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660 Fig. 3

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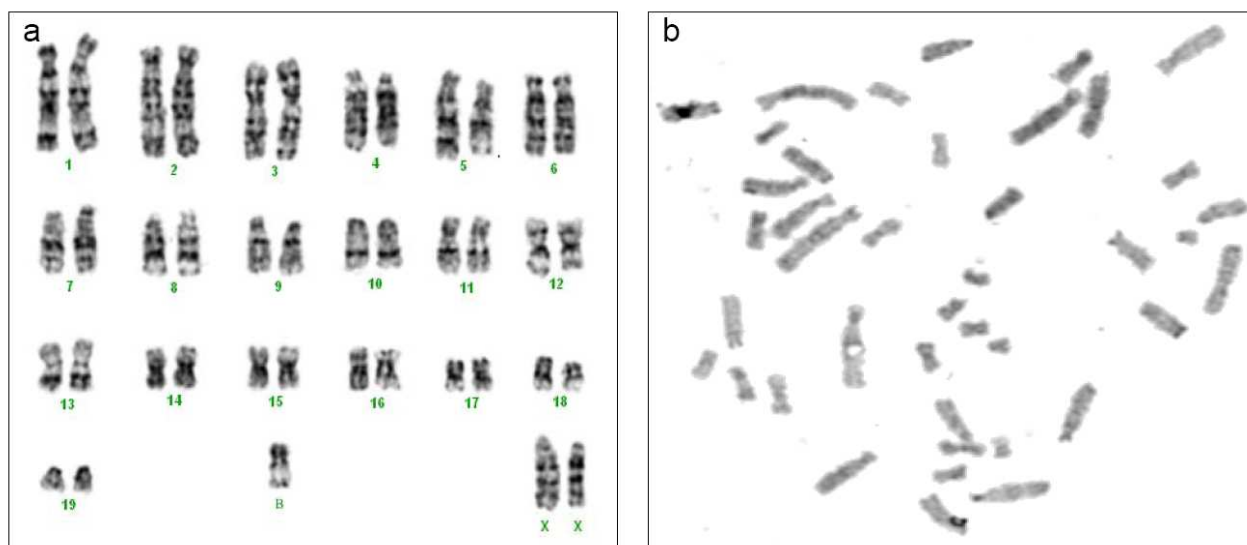
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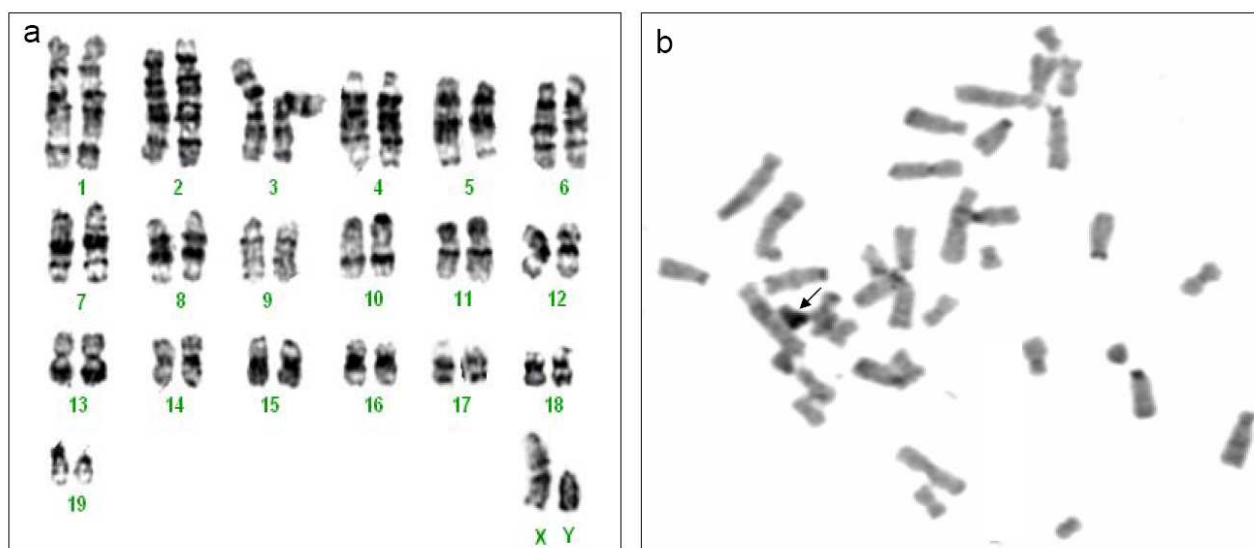
666 Fig. 4

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669 Fig. 5



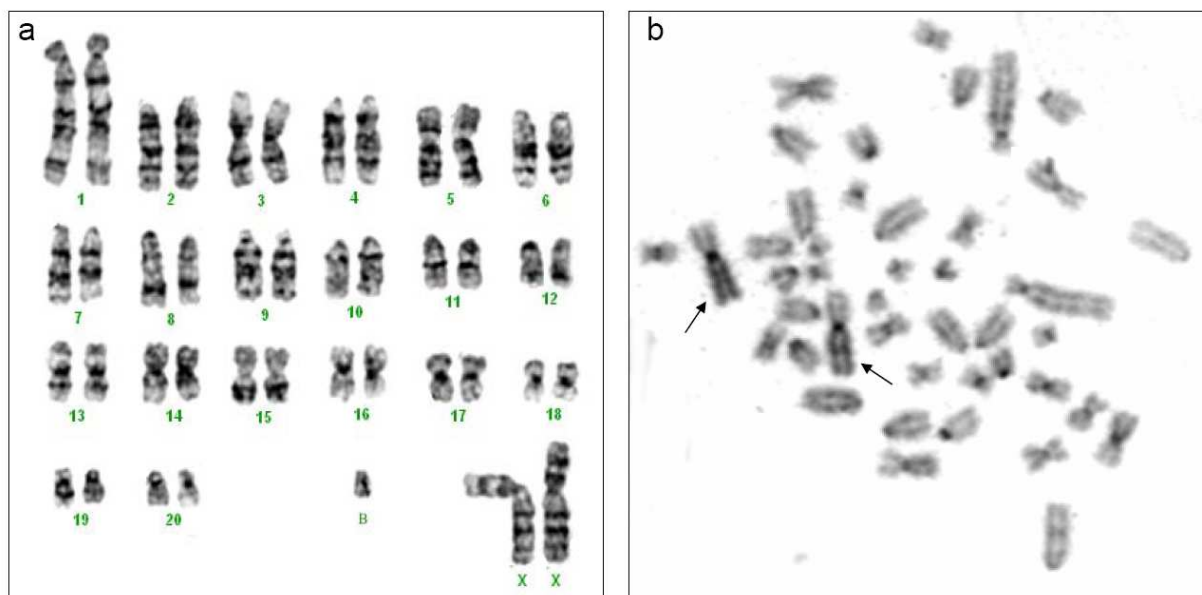
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673 Fig. 6

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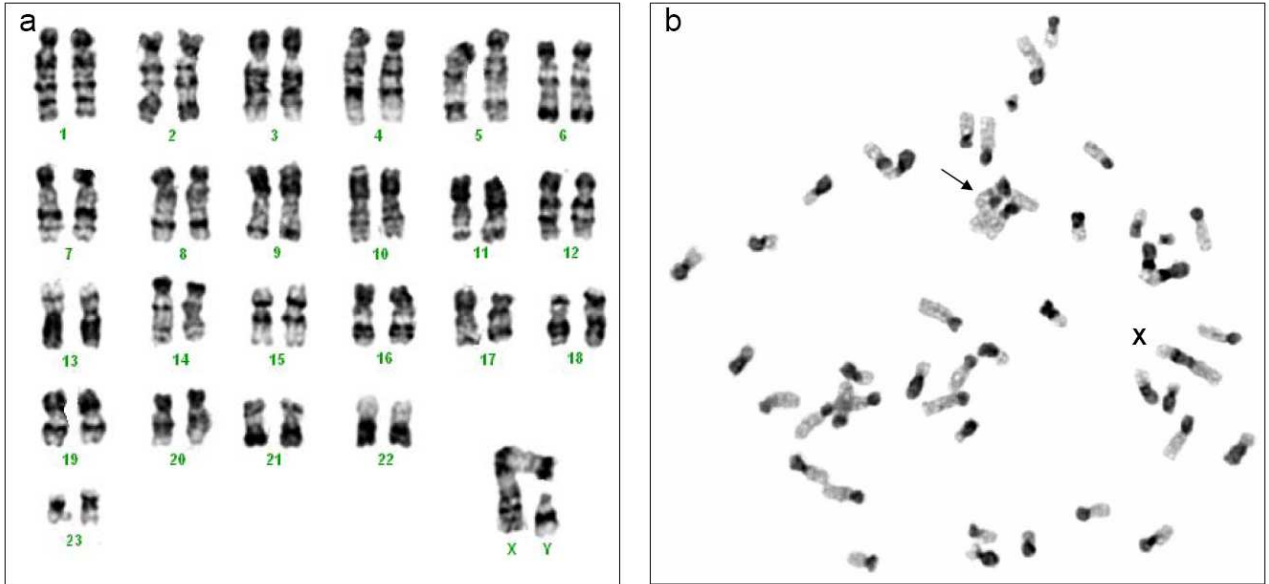


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678 Fig. 7



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681 Fig. 8

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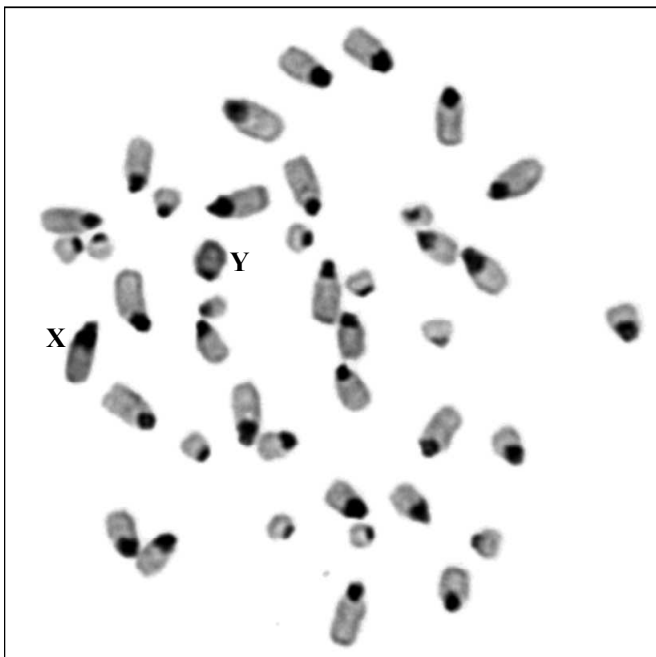
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688 Fig. 9

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