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Variation in chromosome numbers and the sex determination system in the Gerromorpha with special reference to the family Gerridae (Hemiptera)

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1 RESEARCH ARTICLE

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3 **Variation in chromosome numbers and the sex determination system in the**

4 **Gerromorpha with special reference to the family Gerridae**

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22 We assess karyotypic variation in the heteropteran infraorder Gerromorpha.
23 Counts of autosomes, m-chromosomes and sex chromosomes are obtained
24 from literature reports for 51 species, including 35 in the family Gerridae.
25 Placing these data on recently derived phylogenies reveals that XX/X0 sex
26 determination is ancestral to the clade containing the Gerridae and
27 Veliidae, but that XX/XY sex determination has been secondarily derived
28 several times. Our results confirm the absence of m-chromosomes in the
29 Gerromorpha, but contrary to previous reviews, we find intermediate
30 levels of variation in chromosome counts and sex-determining systems
31 when compared to other Heteropteran clades. A new karyological analysis
32 reveals that the X chromosome of *Aquarius remigis* (Say, 1832) is the
33 largest chromosome, which contrasts with *A. najas* (De Geer, 1773) in
34 which it the X chromosome is the smallest. This karyotypic difference
35 supports molecular evidence that the *A. remigis* group of species is not
36 closely related to other species in the genus *Aquarius* Schellenburg, 1800.

37 **Keywords:** Hemiptera; Gerromorpha; Gerridae; karyotype; sex determination

38

39 **Introduction**

40 We report the chromosome number and mechanism of sex determination for 51 species
41 within the heteropteran infraorder Gerromorpha. This report updates previous reviews
42 of karyotypic variation in the Gerromorpha which included only 21 (Ueshima 1979;
43 Andersen 1982) or 34 (Papeschi and Bressa 2006) species. We place the karyotypic
44 information in a phylogenetic context and re-evaluate conclusions derived from the
45 earlier reviews.

46 Gerromorphan karyotypes have previously been characterized as varying little in
47 autosomal chromosome number, having predominantly XX/X0 sex determination and
48 lacking m-chromosomes (*op. cit.*). The latter are atypical chromosomes that are usually

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51 (but not always) minute compared to autosomes and are distinguished by meiotic
52 behavior that differs from both autosomes and sex-chromosomes. Among other
53 peculiarities, they are achiasmatic, as are sex-chromosomes in the Heteroptera, but
54 unlike sex-chromosomes, they undergo reductional separation at the first division and
55 equational separation at the second (for more detailed descriptions of the meiotic
56 behaviors of autosomes, sex-chromosomes and m-chromosomes, see Ueshima (1979)
57 and Papeschi and Bressa (2006)). M-chromosomes are found in a number of other
58 heteropteran clades but have not been found in the Gerromorpha (Ueshima 1979;
59 Papeschi and Bressa 2006; Castanhole et al. 2008; Kuznetsova, Grozeva, Nokkala, and
60 Nokkala 2011; Souza et al. 2014; Pereira et al. 2015).

61 The predominance of XX/X0 sex determination in the Gerromorpha is unusual
62 among heteropteran clades. In the majority of heteropteran species (71%), sex is
63 determined via the familiar XX/XY chromosomal system in which females inherit an X
64 chromosome from each parent whereas males inherit an X chromosome from their
65 mother and a Y chromosome from their father (Ueshima 1979; Papeschi and Bressa
66 2006). However, several variations on this mechanism are also quite common and often
67 co-exist with XX/XY systems within the same taxonomic family (*op. cit.*). The second
68 most common system of sex determination is XX/X0, which occurs in 15% of species.
69 In these species Y chromosomes are absent and the male karyotype is designated as X0,
70 signifying one X chromosome and no Y. The great majority of heteropteran species
71 have either XX/XY or XX/X0 sex determination, but multiple X chromosomes have
72 been found in approximately 13% of species, with some having as many as five X
73 chromosomes. These systems are designated $X_n/X_{n-1}Y$ or $X_n/X_{n-1}0$, with n designating
74 the number of X-chromosomes in females. Multiple Y chromosomes are rare but have
75 been described in two families in the infraorder Pentatomorpha (Papeschi and Bressa

76 2006). Some authors distinguish an additional category of sex-chromosomal variation:
77 the presence of neo-X or neo-Y chromosomes. Neo-sex chromosomes are derived by
78 fusion of ancestral X or Y chromosomes with one chromosome of an autosomal pair.
79 They are rare in the Heteroptera, having been described in only 0.5% of species
80 distributed across in five families (Papeschi and Bressa 2006), including one
81 gerromorphan species in the family Hebridae, *Hebrus pusillus* (Fallén, 1807) (Nokkala
82 and Nokkala 1999).

83 In contrast to the majority of heteropterans, the two early reviews of
84 gerromorphan karyotypes indicated that XX/X0 sex determination (hereafter denoted
85 simply as X0) was almost universal in the Gerromorpha (Ueshima 1979; Andersen
86 1982). The more recent review by Papeschi and Bressa (2006) shows Y chromosomes
87 present in every gerromorphan family but does not provide data at the species level and
88 does not give the prevalence of XY versus X0 sex determination in the Gerromorpha.
89 Ueshima (1979) surmised that X0 is the ancestral sex determination system not only for
90 the Gerromorpha but also for Heteroptera as a whole. If X0 is the ancestral condition
91 for the Gerromorpha, the few occurrences of XY systems or systems with multiple X
92 chromosomes ($X_{n-1}Y$ or $X_{n-1}0$) within the infraorder represent secondary derivations.
93 Subsequent discoveries of Y chromosomes in other gerromorphan species have
94 prompted several authors to question this hypothesis (Calabrese and Talerico 1984,
95 1987; Nokkala and Nokkala 1999). Similarly, documentation of Y chromosomes in
96 basal heteropteran clades has cast doubt on the hypothesis that X0 is the ancestral state
97 for the Heteroptera as a whole (Nokkala and Nokkala 1984; Grozeva and Nokkala
98 1996). We use our expanded data set to re-evaluate the prevalence of X0 sex
99 determination in the Gerromorpha and to determine the ancestral state for the most well
100 represented family, the Gerridae.

101 We also present a new karyologic analysis for the gerrid species *Aquarius*
102 *remigis* (Say, 1832) and estimate of the size of its X chromosome. This species is of
103 particular interest because it is the signature species in the *Aquarius remigis* species
104 group, comprising *A. remigis*, *A. amplus* (Drake and Harris, 1938) and *A. remigoides*
105 (Gallant and Fairbairn, 1996). The taxonomic structure and phylogenetic position of
106 this group have been difficult to discern and its classification remains uncertain (e.g.,
107 Michel 1961; Andersen 1990, 1993, 1995; Damgaard, Andersen, and Sperling 2000;
108 Damgaard 2006; Damgaard, Moriera, Weir, and Zettel 2014). Although still classified
109 in the genus *Aquarius* Schellenburg, 1800, molecular phylogenetic analyses now place
110 these species as more closely related to *Gerris* species than to other *Aquarius* species
111 (Damgaard and Cognato 2006; Damgaard and Christiansen 2007; Damgaard 2012;
112 Damgaard et al. 2014). Based on its phenotypic and genetic distance from other
113 *Aquarius* species and from *Gerris* species, Damgaard has recommended that the *remigis*
114 group should be classified as a separate genus, but this taxonomic change has not yet
115 been implemented (Damgaard and Christiansen 2007; Damgaard et al. 2014). The
116 signature species, *A. remigis*, is itself problematic from a taxonomic perspective. It is
117 one of the most widely distributed and phenotypically variable of the temperate gerrids
118 (Michel 1961; Calabrese 1974; Andersen 1990; Brennan and Fairbairn 1995; Fairbairn
119 2005; Damgaard and Christiansen 2007; Fairbairn and King 2009), and populations
120 show marked genetic differentiation both within and among regions (Zera 1981;
121 Preziosi and Fairbairn 1992; Gallant, Preziosi, and Fairbairn 1993). Populations from
122 California and Oregon are particularly distinct with respect to genital morphology, body
123 color, life history and flight propensity (Michel 1961; Calabrese 1974; Fairbairn and
124 King 2009; Fairbairn, personal observations). Michel (1961) considered these western
125 populations a separate species and Calabrese (1974) gave them subspecific status.

126 However more recent comparisons of allozyme frequencies and of the shape of the
127 internal genital components among populations from across North America have not
128 supported separate taxonomic classification of western *A. remigis* (Preziosi and
129 Fairbairn 1992; Gallant et al. 1993; Damgaard and Christiansen 2007), and all *A.*
130 *remigis* are still considered a single species (Polhemus and Chapman 1979; Andersen
131 1990; Damgaard and Christiansen 2007; Damgaard et al. 2014). The phylogenetic
132 position and taxonomic status of the *Aquarius remigis* group and of its signature species
133 therefore remain a key issue in fully resolving the phylogeny of the Gerridae.

134 In addition to this significance for gerromorphan systematics, *Aquarius remigis*
135 is a model species for studying dispersal polymorphisms (e.g., Fairbairn 1988; Fairbairn
136 and King 2009; Kaitala and Dingle 1992, 1993), multilevel selection (Eldakar, Wilson,
137 Dlugos, and Pepper 2010; Chang and Sih 2013), and sexual selection and sexual
138 dimorphism (e.g., Fairbairn 1992, 2005, 2007; Sih and Krupa 1995; Fairbairn and
139 Preziosi 1994, 1996; Watson, Arnqvist, and Stallmann 1998; Preziosi and Fairbairn
140 2000; Sih, Lauer, and Krupa 2002; Bertin and Fairbairn 2005). In the latter context, a
141 recent study quantifying the effect of sex-linked genes on body size and sexual
142 dimorphism has underlined the need to verify the sex-determination mechanism in this
143 species and to estimate the relative size of the X chromosome (Wolak 2013).

144 Calabrese and Tallerico (1982) published a karyotype for *A. remigis* from
145 Aguirre Springs, New Mexico but they did not assess the relative size of the X
146 chromosome. Our intent is to measure the relative size of the X chromosome and also
147 to determine if the chromosome count and sex determination system for *A. remigis* from
148 California match those of the New Mexican population. Differences between the
149 karyotypes of Californian *A. remigis* and those from New Mexico would suggest a
150 reassessment of the taxonomic status for populations from the western region.

151

152 **Material and methods**

153 *Aquarius remigis* are surface-dwelling, semi-aquatic predators found on streams and
154 small rivers throughout much of temperate and subtropical North America and
155 Mesoamerica (Calabrese 1974; Andersen 1990; Preziosi and Fairbairn 1992; Gallant et
156 al. 1993; Damgaard and Christiansen 2007). They are among the largest of the
157 temperate gerrids, ranging in length from about 11–17 mm (Brennan and Fairbairn
158 1995). As is typical of water striders, they subsist primarily by capturing insects and
159 other small arthropods that fall on the water surface and become trapped in the surface
160 film. Female *A. remigis* oviposit on submerged, solid surfaces, but the newly hatched
161 nymphs swim to the surface and all five nymphal stages live on the water surface. Most
162 populations have one or two generations per year and overwinter as adults in
163 reproductive diapause (Fairbairn 1985; Blanckenhorn 1991; Blanckenhorn and
164 Fairbairn 1995). However, low altitude populations in Southern California have a more
165 flexible reproductive pattern and laboratory cultures kept under long day conditions
166 (14L:10D) maintain continuous reproduction.

167 For our karyologic analysis of *A. remigis* we used the testes of reproductively
168 mature, adult males from a laboratory culture maintained under 14L:10D at the
169 University of California, Riverside. The males sampled were first or second generation,
170 lab-reared descendants of adults captured from natural populations in western San
171 Bernardino County, California. Each male was anesthetised with chloroform prior to
172 dissection. The four testes were then removed and immediately placed in modified
173 Carnoy fixative (3:1 absolute ethanol : glacial acetic acid) for minimum 48 hours and
174 kept at 4°C. After being fixed, the testes were stained with 2% solution of aceto-orcein
175 for 3–4 days (Kiseliova, Toderas, and Derjanschii 1998) and then squashed in a drop of

176 45% acetic acid. Squashed preparations were scanned immediately under oil immersion
177 using an OlympusTMBX51 (DIC) microscope. Microphotographs of mitotic and
178 meiotic metaphase plates were taken with a Sony HDR-HC1K camera. Our karyotypic
179 interpretations are based on 90 metaphase plates prepared from 38 males.

180 As is typical of Hemipteran insects (e.g., Thomas 1987; Papeschi and Bressa
181 2006; Melters, Paliulis, Korf, and Chan 2012; Bardela, Gil-Santana, Panzera, and
182 Vanzela 2014; Souza et al. 2014; Pereira et al. 2015), all species in the Gerromorpha
183 have holocentric chromosomes that are globular in shape and lack a localized
184 centromere (Ueshima 1979; Andersen 1982; Calabrese and Tallerico 1982, 1984, 1987;
185 Newman and Cheng 1983; Spence and Maddison 1986; Grozeva, Nokkala, and Simov
186 2009; Castanhole 2009). This makes it difficult to discern distinct characteristics of
187 individual chromosomes other than size. However, the sex chromosomes can be
188 distinguished from autosomes because they undergo equational rather than reductional
189 division during meiotic metaphase I and hence do not form a bivalent during this phase
190 (Ueshima 1979; Spence and Maddison 1986; Papeschi and Bressa 2006). We estimated
191 the relative size of the X chromosome for 10 male *A. remigis* from meiotic metaphase I
192 plates in which all of the chromosomes were separated clearly enough for accurate
193 measuring. Using ImageJ software (Rasband 2015), we measured the area of all 21
194 chromosomes and summed these areas to get a total chromosome area. The relative
195 size of the X chromosome was then estimated as the area of the X chromosome divided
196 by the total chromosome area. We also ranked the 21 chromosomes from largest to
197 smallest by area and determined the rank of the X chromosome for each male.

198 To assess the phylogenetic history of sex determining systems within the
199 Gerridae and to place the karyotype of *A. remigis* in its phylogenetic context, we
200 mapped species' chromosome counts and sex-chromosome systems onto a phylogeny of

201 the subfamily Gerrinae derived from Damgaard et al. (2014). We trimmed the original
202 tree to include only species with known karyotypes, and with two exceptions, we
203 included only the species used by Damgaard et al. (2014). The two exceptions are
204 species used as outgroups for the subfamily Gerrinae. Damgaard et al. (2014) used
205 *Cylindrostethus costalis* (Schmidt, 1915) to represent the subfamily Cylindrostethinae
206 and *Brachymetra unca* (Shaw, 1933) to represent the subfamily Charmatometrinae, but
207 we could not include these species because their karyotypes have not been determined.
208 We, therefore, substituted their congeners, *Brachymetra albinerva* (Amyot and Serville,
209 1843) and *Cylindrostethus palmaris* (Drake and Harris, 1934) to represent these
210 subfamilies. To assess the history of sex determining systems more broadly in the
211 Gerromorpha, we mapped the frequency of sex determining systems (X0, XY and X_n-
212 ₁Y) on a phylogeny of gerromorphan families and subfamilies derived from Damgaard
213 (2008, 2012). As above, we trimmed the tree to include only families containing
214 species with known karyotypes.

215

216 **Results**

217 Our karyologic study of *Aquarius remigis* verifies the earlier conclusions of Calabrese
218 and Talerico (1982). Photomicrographs of mitotic metaphase in spermatogonia and of
219 meiotic metaphase I in primary spermatocytes consistently revealed a karyotype
220 consisting of 21 chromosomes: ten pairs of autosomes plus a single X chromosome
221 (Figure 1a, 1b). Thus, the diploid karyotype of male *A. remigis* is $2n = 20A + X0$. Also
222 in agreement with Calabrese and Talerico (1982), we found no evidence of any m-
223 chromosomes.

224

{FIGURE 1a AND 1b NEAR HERE}

225 The X chromosome of *A. remigis* was consistently the largest chromosome. It
226 ranked first by area in each of the 10 males measured and averaged 12.9% (SD 5.8%)
227 larger than the largest autosome. The difference in size between the X chromosome and
228 the largest of the autosomes was highly statistically significant ($t_{2-tailed} = 6.300$, df 9, P
229 < 0.001). By area, the X chromosome comprised an average of 7.4% (SD 0.4%) of the
230 diploid chromosome complement of males; by extension, two X chromosomes would
231 comprise 13.8% of the diploid chromosome complement in females. These values
232 compare to expected values based on chromosome counts of $1/21 = 4.8\%$ in males and
233 $2/22 = 9.1\%$ in females.

234 To assess the distribution of chromosome counts and sex determination systems
235 in the Gerromorpha, we assembled literature reports for 51 species, including 30 species
236 not listed in Ueshima (1979) or Andersen (1982). We also found reports of seven new
237 analyses for previously reported species. Most of the duplicate analyses confirmed
238 previous reports, but we found conflicts for three species. For *Gerris marginatus* (Say,
239 1832), Ueshima (1979) reported a male chromosome count of $2n = 20A + X0$, citing
240 Montgomery (1901), and Andersen (1982) repeated this, citing Ueshima. However,
241 Calabrese and Talerico (1982) reported a chromosome count of $2n = 18A + XY$ for the
242 same species. They argued that Montgomery may have been studying a different
243 species because in 1901 at least three currently recognized species were included under
244 the name *G. marginatus*. Given this taxonomic uncertainty, we use the karyotype
245 reported by Calabrese and Talerico for *G. marginatus*. Unfortunately, conflicting
246 reports for two other species could not be similarly resolved. Ueshima (1979), followed
247 by Andersen (1982), reported a chromosome count of $2n = 22A + X0$ for *Aquarius*
248 *paludum* (Fabricius, 1794), citing a series of papers by Wilke (1907, 1912, 1913). This
249 karyotype is for the subspecies *Aquarius paludum paludum* (Fabricius, 1794) which

250 occurs throughout the Palearctic region. Ueshima (but not Andersen) also listed the
251 karyotype $2n = 22A + XY$ reported by Takenouchi and Muramoto (1968) for *A.*
252 *paludum*. This karyotype may be for the subspecies *A. p. amamiensis* (Miyamoto,
253 1958) which occurs only in Japan. Although an early comparison based on the
254 mitochondrial gene cytochrome oxidase subunit I (COI) could not distinguish *A. p.*
255 *amamiensis* from *A. p. paludum* (Damgaard and Zettel 2003), more recent consensus
256 analyses based on both genetic and morphological characters (Damgaard and Cognato
257 2006) or additional genetic sequences alone (Damgaard et al. 2014) do clearly separate
258 the two subspecies. Because both subspecies occur in Japan (Damgaard et al. 2014) and
259 the subspecific status of the sample used by Miyamoto (1958) is not certain, we include
260 both karyotypes under the species name *A. paludum*. We were similarly unable to
261 resolve the conflicting reports for *Hebrus ruficeps* (Thomson, 1871). Ueshima (1979)
262 and Andersen (1982) report a chromosome count of $2n = 18A + X0$ for males of this
263 species, citing Cobben (1968). However, Nokkala and Nokkala (1999) report a
264 karyotype of $22A + XY$ for the same species. The latter authors note that their
265 observations differ from those of Cobben, but provide no explanation for the disparity.
266 We, therefore, report both karyotypes. Because the karyotypic status remains uncertain
267 for both *H. ruficeps* and *Aquarius paludum*, we do not include either species in our
268 estimates of the prevalence of sex determining systems and we exclude *Hebrus ruficeps*
269 from the summary of autosomal chromosome numbers.

270 Our compilation of karyotypes reveals diploid autosome counts ranging from 18
271 to 38 in the Gerromorpha (Figure 2). Counts of 18 and 20 are most prevalent and
272 equally common, with 13 species each, and the second most common category is 22,
273 with 12 species. Together these categories comprise more than three quarters (76%) of
274 the known gerromorphan karyotypes. Among the Gerridae, the modal diploid

275 autosomal count is 20 with a range of 18 to 30, and 80% of species have counts of 18,
276 20 or 22.

277 {FIGURE 2 NEAR HERE}

278 The majority of species have X0 sex determination but this mechanism is far
279 from universal (Table 1, Figures 3, 4). Eleven species have XY sex determination,
280 including seven within the Gerridae, and one species in the family Mesovelliidae has
281 five X chromosomes and X₄Y sex determination (Ekholm 1941; Grozeva et al. 2009).
282 As noted above, there are also conflicting reports of both XY and X0 for *Aquarius*
283 *paludum* in the Gerridae and for *Hebrus ruficeps* in the Hebridae. Thus, Y
284 chromosomes occur in at least 12 and possibly 14 species representing about a quarter
285 (23.5%–27.5%) of the reported gerromorphan karyotypes. Within the Gerridae, the
286 prevalence of XY sex determination is between 20% (7/35) and 22.9% (8/35). By
287 comparison, the prevalence of Y chromosomes among species from other gerromorphan
288 families is between 31.3% (5/16) and 37.5% (6/16). This suggests a slightly lower
289 prevalence of Y chromosomes in the Gerridae, but the difference is far from statistically
290 significant ($X^2 = 0.887$, df 2, $P > 0.25$).

291 {FIGURE 3 NEAR HERE}

292 Figure 3 places the sex determining systems on a phylogeny of the most well-
293 represented subfamily, the Gerrinae, with species from two other subfamilies within
294 Gerridae and its sister family, Veliidae, as outgroups. This phylogenetic representation
295 indicates that the ancestral condition for the Gerrinae is X0 and that there have been at
296 least two transitions to XY. One transition occurred on the branch leading to the clade
297 containing *Gerris comatus* (Drake and Hottes, 1925a), *G. marginatus*, *G. insperatus*
298 (Drake and Hottes, 1925b) and *G. alacris* (Hussey, 1921), and another occurred on the
299 branch leading to *Limnporus canaliculatus* (Say, 1832). If *Aquarius paludum* is XY as

300 reported by Takenouchi and Muramoto (1968), a third transition must also have
301 occurred on the branch leading to this species.

302 {FIGURE 4 NEAR HERE}

303 In Figure 4, the frequencies of X0, XY and $X_{n-1}Y$ sex determination are shown
304 on a phylogeny of subfamilies within the Gerridae -Veliidae clade and within three
305 other gerromorphan families: Hydrometridae, Hebridae and Mesoveliidae (Damgaard
306 2008, 2012). The analysis within the Gerrinae (above) established that XY systems are
307 derived within this subfamily, and this combined with the absence of XY in the five
308 species of Halobatinae (the sister group of all other Gerridae), suggests that X0 sex
309 determination is the ancestral state in the Gerridae as a whole. If this is correct, the
310 species with XY sex determination in the subfamilies Trepobatinae and the
311 Rhagadotarsinae represent independent derivations. Although sampling is sparse
312 outside of the Gerridae, X0 systems predominate in the three veliid subfamilies as well
313 as in the clade containing the families Hydrometridae and Hebridae. These
314 observations further support the hypothesis that X0 sex determination is the ancestral
315 state for the family Gerridae.

316

317 **Discussion**

318 The chromosome complement of $2n = 20A + X0$ that we observed for male *Aquarius*
319 *remigis* is typical of species in the family Gerridae with respect to both the number of
320 autosomes and the sex-determination system. It also agrees with the previously
321 published karyotype for this species from New Mexico (Calabrese and Tallerico 1982)
322 and so does not provide evidence supporting separate subspecific or specific status for
323 Californian *A. remigis*. Because the chromosome counts and the sex-determination
324 system are so conserved in the Gerrinae, the *A. remigis* karyotype is not informative

325 with respect to its phylogenetic position as a sister clade of *Gerris* (Figure 3; Damgaard
326 et al. 2014). Nor does our new information contribute meaningfully to consideration of
327 new generic status for *A. remigis* and its sister species, *A. remigoides* and *A. amplus*
328 (Damgaard et al. 2014).

329 Few authors have reported the relative size of the X chromosome in
330 gerromorphan species and published photographs are often not of sufficient resolution
331 for this to be assessed. Nevertheless, the available examples suggest considerable
332 interspecific variation (Andersen 1982). The X chromosome is the largest or one of the
333 largest chromosomes in *Gerris costae* (Herrich-Schäffer, 1950) (Grozeva et al. 2009)
334 and *G. gibbifer* (Schummel, 1982) (Poisson 1936); of average or intermediate size in *G.*
335 *asper* (Fieber, 1860), *G. lateralis* (Schummel, 1832), *Limnoporus notabilis* (Drake and
336 Hottes, 1925a) and *Tachygerris opacus* (Champion, 1898) (Ekblom 1939; Spence and
337 Maddison 1986; Callebresse and Talerico 1987); one of the smallest chromosomes in
338 *Limnogonus aduncus* (Drake and Harris, 1933) (Castanhole et al. 2008); and the
339 smallest in *Aquarius najas* (Poisson, 1936). Within the genus *Gerris* Fabricius, 1794,
340 the two species with large X chromosomes are closely related and in a different clade
341 than the two with intermediate X chromosomes, and the latter are sister species (Figure
342 3; Damgaard et al. 2014). This clustering suggests that the relative size of the X
343 chromosome may show phylogenetic patterning. If so, the stark contrast between
344 *Aquarius najas*, where the X chromosome is the smallest, and *A. remigis*, where it is the
345 largest by a significant margin, supports Damgaard's evidence that *A. remigis* and *A.*
346 *najas* are not closely related, and that *A. remigis* should be assigned to a new genus.

347 As discerned in previous reviews, the XO sex-determination system clearly
348 predominates in the Gerromorpha. Of the 51 species for which we have data, at least 37
349 (72.5%) and possibly as many as 39 (76.5%) have XO sex determination. All other

350 species have XY sex determination with the exception of *Mesovelgia furcata* (Mulsant
351 and Rey, 1852) in which males are X₄Y (Grozeva et al. 2009). The prevalence of X0
352 sex determination may be slightly higher in the Gerridae than in the other families
353 sampled, but previous observations of universal X0 in the Gerridae (Ueshima 1979, p.
354 92; Andersen 1982, p. 56) are not supported by the new data.

355 The high prevalence of X0 sex determination in the Gerromorpha is unusual in
356 the Heteroptera. In a review of chromosomal sex determination across 1145
357 heteropteran species in 42 families, Ueshima (1979, pp. 91–99) found X0 sex
358 determination in only 15.1% of species whereas 73.9% were XY. The remainder had
359 multiple X chromosomes with (8.3%) or without (2.7%) a Y, or multiple Y
360 chromosomes (one species). A more recent survey of 1600 heteropteran species in 46
361 families found similar proportions: 14.7% X0, 71.4% XY, and 13.5% with multiple X's
362 or multiple Y's (one species) (Papeschi and Bressa 2006). The remaining 0.5% were
363 categorized as neo-sex chromosome systems and the authors did not specify either the
364 numbers or the types of sex chromosomes for these species. Y-chromosomal systems
365 also predominate at the family level: Y chromosomes are universal in 24 families
366 (53.3%), occur in a portion of species in 16 families (35.6%), and are entirely absent
367 from only five families (11.1%) (Papeschi and Bressa 2006).

368 In spite of the prevalence of Y-chromosomal systems in the Heteroptera,
369 Ueshima (1979) argued that X0 sex determination is the ancestral state for the entire
370 suborder, as well as for the infraorder Gerromorpha. Several authors have subsequently
371 questioned this hypothesis, based on findings of XY systems in additional
372 gerromorphan species (Calabrese and Talerico 1984, 1987; Nokkala and Nokkala
373 1999) as well as in more basal heteropteran families (Nokkala and Nokkala 1984;
374 Grozeva and Nokkala 1996). Our phylogenetic reconstructions cannot address the

375 question of the ancestral state for the Heteroptera or even for the Gerromorpha.
376 However, our data do clearly support the hypothesis that X0 is the ancestral state for the
377 subfamily Gerrinae, the family Gerridae, and most likely also for the clade containing
378 the Gerridae plus Veliidae. The eight (or nine) XY systems found within this clade
379 appear to have evolved through five (or six) independent evolutionary transitions from
380 the ancestral X0 state.

381 Andersen (1982) characterized the variation in chromosome number in the
382 Gerromorpha as ‘quite insignificant’. While this may be an exaggeration (see below),
383 our more extensive compilation of species does reveal relatively little variation not only
384 in the sex-determining system, but also in the numbers of autosomes. Our observation
385 that 76% of reported diploid autosomal counts are between 18 and 22 supports this
386 characterization for the infraorder as a whole, and variation is particularly low in the
387 most well-characterized family, the Gerridae, where 80% of species have between 18
388 and 22 autosomes. Nevertheless, our compilation has considerably broadened the range
389 of diploid autosomal chromosome counts reported for this infraorder. Ueshima (1979),
390 Andersen (1982) and Papeschi and Bressa (2006) reported a range of between 18 and
391 30. In our compilation, the lower limit remains 18, but a recent report of the karyotype
392 of *Rhagovelia whitei* (Breddin, 1898) extended the upper limit to 38 (Castanhole,
393 Pereira, Souza, and Itoyama 2012). The modal autosome count for the species listed in
394 Ueshima (1979) and Andersen (1982) was 20, but our expanded sampling has extended
395 this to include both 18 and 20. Within the Gerridae, however, the mode remains at 20.

396 Our compilation also supports the conclusion of previous reviewers (Ueshima
397 1979; Andersen 1982; Papeschi and Bressa 2006) that m-chromosomes are absent from
398 the Gerromorpha. These enigmatic chromosomes have been found in at least 14
399 families in the Heteroptera, including two of the most speciose (Coriidae and

400 Lygaeidae) and several aquatic families (Corixidae, Naucoridae, Notonectidae and
401 Pleidae) (Ueshima 1979; Papeschi and Bressa 2006). It is premature to conclude that
402 m-chromosomes will not be found in more broadly across the Heteroptera as more
403 karyotypes are resolved. However, their absence from the species included in our
404 survey is strong evidence that they are absent from the Gerridae and supports the
405 prediction that they are also absent throughout the Gerromorpha.

406 Relative to other families in the Heteroptera, the variation in chromosome
407 counts and sex-determination systems that we found in the Gerridae and in our limited
408 samples of other gerromorphan families is neither unusually low nor unusually high.
409 Many families have much lower karyotypic variation than apparent in Gerromorphan
410 families. Examples include the Rhopalidae which consistently have $2n = 13$ or 15 with
411 two m-chromosomes and X0 sex determination; the Scutelleridae with $2n = 12-14$, no
412 m-chromosomes, and XY sex determination; and the Corixidae with $2n = 24-26$, one
413 pair of m-chromosomes and XY sex determination (Ueshima 1979; Papechi and Bressa
414 2006; Souza et al. 2014). At the other extreme, many families show much more
415 variation in both the numbers of chromosomes and their distribution among categories
416 (i.e., autosomes, sex chromosomes and m-chromosomes) than we found in the
417 Gerromorpha (Ueshima 1979; Thomas 1987; Papeschi and Bressa 2006; Castanhole et
418 al. 2008; Kuznetsova et al. 2011; Souza et al. 2014; Kaur and Gaba 2015). For
419 example, species in the Lygaeidae have diploid chromosome counts varying from $2n =$
420 $10-30$, may or may not have m-chromosomes, and have many different sex-determining
421 systems including X0, XY, $X_{n-1}Y$ and XY_n (Ueshima 1979; Papeschi and Bressa 2006).
422 Several subfamilies of Lygaeids show almost this full range of karyotypic variation
423 (Ueshima 1979). Similarly, in the Coreidae, the diploid karyotype varies from $2n = 13$
424 to 28 , and the sex-determining system can be X0, $X_{n-1}O$ or XY with almost the full

425 range of variation occurring within a single subfamily, the Corinae (Papeschi and
426 Bressa 2006; Souza et al. 2014). Among aquatic Heteroptera, the most variable families
427 are: the Belostomatidae with chromosome counts varying from $2n = 4-30$ and XY, X_n-
428 $_1Y$ and neo-XY sex-determination systems; and the Nepidae with $2n$ varying from 22–
429 46 and X0, XY or $X_{n-1}Y$ sex determination (Papeschi and Bressa 2006). Clearly, the
430 rate of karyotypic evolution has been highly labile both within and among heteropteran
431 families. Discerning the evolutionary causes and functional significance of the
432 variation in heteropteran karyotypes and their rate of evolution remains a challenge for
433 future research.

434

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446

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449

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778

779 **Table 1:** Diploid autosomal chromosome counts and sex chromosomes for males of 27 species from five families in the infraorder Gerromorpha.

780 Data for an additional 24 species in the family Gerridae, subfamily Gerrinae are shown in Figure 3.

FAMILY, subfamily	Species	Number of autosomes	Male sex chromosomes	Source
GERRIDAE				
Charmatometrinae	<i>Brachymetra albinerva</i> (Amyot and Serville, 1943)	24	XO	Castanhole et al. (2010)
Cylindrostethinae	<i>Cylindrostethus palmaris</i> (Drake and Harris, 1934)	28	XO	Castanhole et al. (2010)
Halobatinae	<i>Asclepios shiranui</i> (Esaki, 1924)	22	XO	Newman and Cheng (1983)
	<i>Halobates flaviventris</i> Eschscholtz, 1822	30	XO	Newman and Cheng (1983)
	<i>H. germanus</i> White, 1883	30	XO	Newman and Cheng (1983)
	<i>H. micans</i> Eschscholtz, 1822	30	XO	Newman and Cheng (1983)
	<i>H. robustus</i> Barber, 1925	30	XO	Newman and Cheng (1983)
Rhagadotarsinae	<i>Rheumatobates rileyi</i> Bergroth, 1892	18	XY	Calabrese and Tallerico (1984)
	<i>R. crassifemur</i> Esaki, 1926	20	XO	Pereira et al. (2015)
Trepobatinae	<i>Halobatopsis platensis</i> Berg, 1879	24	XO	Castanhole et al. (2010)
	<i>Metrobates hesperius</i> Uhler, 1871	18	XY	Calabrese and Tallerico (1984)
VELIIDAE				
Microveliinae	<i>Hebrovelia</i> sp.	20	XO	Ueshima (1979)
	<i>Microvelia douglasi</i> Scott, 1874	20	XY	Ueshima (1979)
Rhagoveliinae	<i>M. reticulata</i> (Burmeister, 1835)	20	XO	Ueshima (1979); Grozeva et al. (2009)
	<i>Rhagovelia becki</i> Drake and Harris, 1936	18	XY	Onopa (1981)
	<i>R. tenuipes</i> Champion, 1898	22	XO	Pereira et al. (2015)
	<i>R. whitei</i> Breddin, 1898	38	XO	Castanhole et al. (2012)
	<i>R. zela</i> Drake, 1959	22	XO	Pereira et al. (2015)
Veliinae	<i>Velia caprai</i> Tamanini, 1947	24	XO	Ueshima (1979)
	<i>V. pelagonensis</i> Hoberlandt, 1941	24	XO	Grozeva et al. (2009)

	<i>Merragata hebroides</i> White, 1877	26	XO	Ueshima (1979)
HYDROMETRIDAE				
Hydrometrinae	<i>Hydrometra gracilenta</i> Horváth, 1899	18	XO	Grozeva et al. (2009)
	<i>H. lineata</i> Eschscholtz, 1822	18	XO	Ueshima (1979)
	<i>H. procera</i> Horváth, 1905	18	XY	Ueshima (1979)
MESOVELIIDAE				
Mesoveliinae	<i>Mesovelia furcate</i> Mulsant and Rey, 1852	30	X ₄ Y	Ueshima (1979); Grozeva et al. (2009)

781 **Figure Captions**

782

783 **Figure 1:** Chromosomes from metaphase plates of spermatogonial cells and primary
784 spermatocytes of males of *Aquarius remigis* (Say, 1832): (A) mitotic metaphase in
785 spermatogonia showing the male diploid chromosome number $2n = 21$; (B) meiotic
786 metaphase I in primary spermatocytes showing 10 pairs of autosomes and a single X
787 chromosome (indicated by the arrow). Scale bar 10 μm .

788

789 **Figure 2:** The distribution of autosomal chromosome counts for 50 species in the
790 infraorder Gerromorpha. Counts for species in the family Gerridae are indicated by
791 black bars. *Hebrus ruficeps ruficeps* Thomson, 1871 (family Hebridae) is excluded
792 because of conflicting reports of autosomal chromosome counts (see Table 1).

793

794 **Figure 3.** Diploid autosomal chromosome numbers and sex chromosomes for males
795 from 24 species and six genera in the family Gerridae, subfamily Gerrinae. Species
796 from two other subfamilies within the Gerridae (Charmatometrinae and
797 *Cylindrostethinae*) and from the family Veliidae (sister clade to the Gerridae) are
798 included as outgroups. The cladogram is adapted from Damgaard et al. (2014) and is a
799 consensus phylogeny based on parsimony analysis of 2268 bp of DNA sequence data
800 from genes encoding COI+II, 16S rRNA and 28S rRNA. Only species with known
801 karyotypes are shown. Arrowheads denote transitions (black) or possible transitions
802 (grey) from XX/X0 to XX/XY sex determination. Karyotype descriptions follow
803 Ueshima (1979) and Andersen (1982), except where noted by superscripts. Additional
804 sources are: ^aGrozeva et al. (2009), ^bCalabrese and Talerico (1982), ^cpresent study,
805 ^dTakenouchi and Muramoto (1968; as noted in Ueshima 1979 and Calabrese and
806 Talerico 1982), ^eSpence and Maddison (1986), ^fCalabrese and Talerico (1984),

807 ^gCalabrese & Talerico (1987), ^hCastanhole et al. (2008), ⁱCastanhole, Pereira, Souza,
808 and Itoyama (2010).

809

810 **Figure 4:** Numbers of species with XX/X0, XX/XY and other sex determination
811 systems by subfamily and family within the infraorder Gerromorpha. The cladogram is
812 adapted from Damgaard (2008, 2012) and is based on a parsimony analysis of 64
813 morphological characters and 2.5 kb of DNA sequence data from the mitochondrial
814 genes cytochrome c oxidase subunit I + II (COI + II) and large mitochondrial ribosomal
815 subunit (16S rRNA) and the nuclear gene large nuclear ribosomal subunit (28S rRNA).
816 Only subfamilies containing species with known karyotypes are shown.

Figure 1a

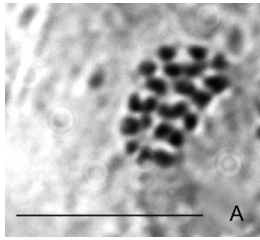


Figure 1b

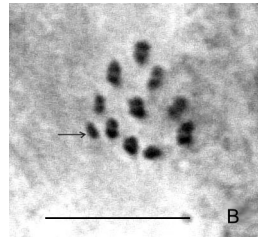


Figure 2

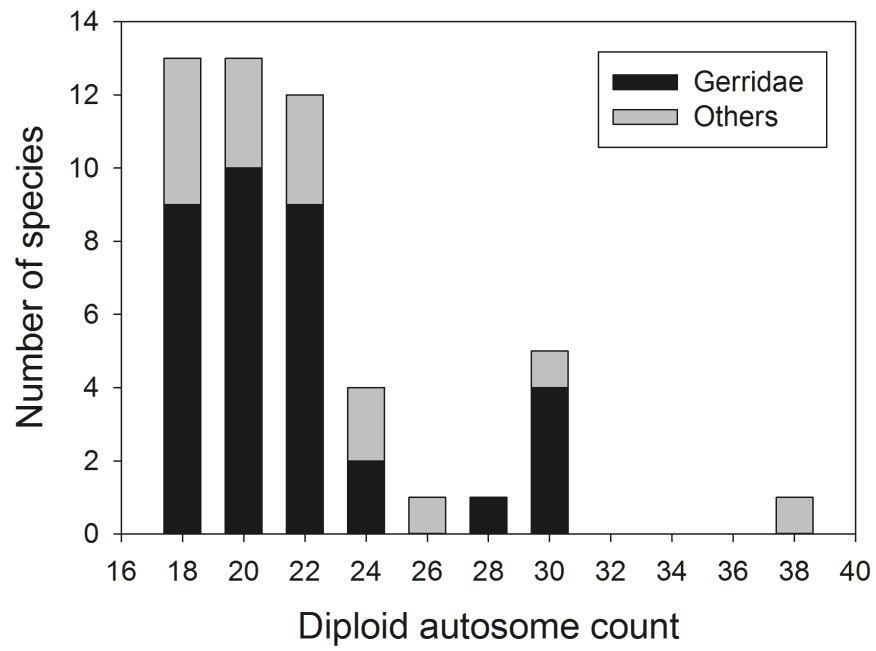


Figure 3

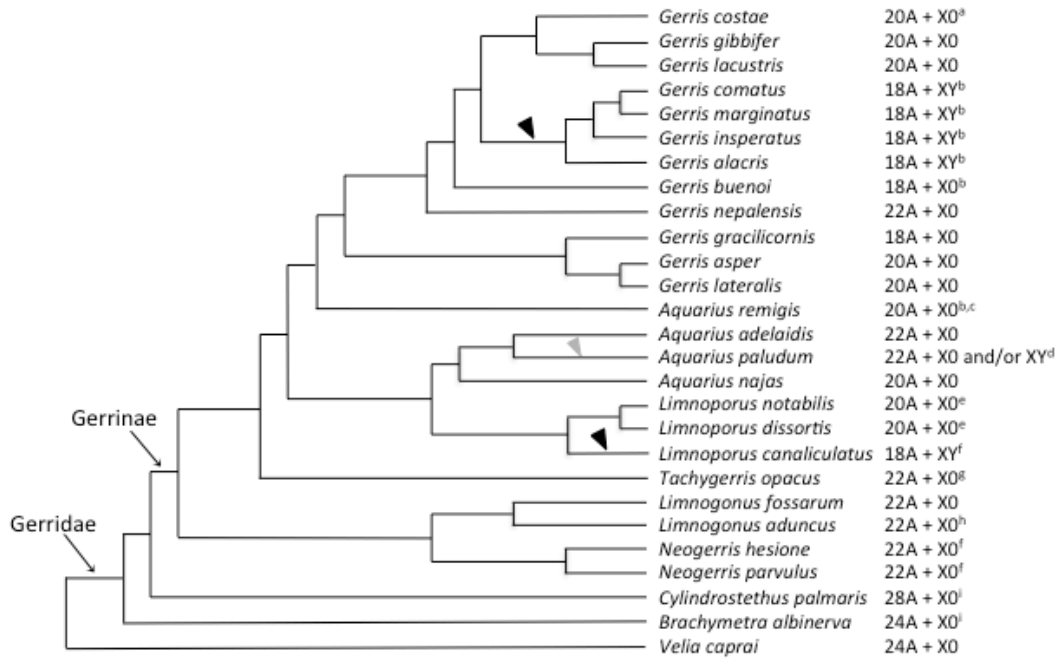


Figure 4

