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Karyotypes of water scavenger beetles (Coleoptera: Hydrophilidae): new data and review of published records

ROBERT B. ANGUS[1], DAVID SADÍLEK[2], FATMA SHAARAWI[3], HAYLEY DOLLIMORE[4‡], HSING-CHE LIU[5], MATTHIAS SEIDEL[6], VÍT SÝKORA[2] and MARTIN FIKÁČEK[2,7*]

[1]*Department of Life Sciences (Insects), Natural History Museum, Cromwell Road, London SW7 5 BD, UK*
[2]*Faculty of Science, Charles University, Viničná 7, CZ-128 43, Praha 2, Czech Republic*
[3]*Department of Entomology, Ain Shams University, Abbassia, Cairo, 11566, Egypt*
[4]*2 Timber Yard, Bradwell Grove, Burford, Oxfordshire OX18 4JR, UK*
[5]*Department of Environmental Engineering and Management, Chaoyang University of Technology, No.168, Jifeng E. Road, Taichung City 413, Taiwan*
[6]*Centrum für Naturkunde, University of Hamburg, Martin-Luther-King Platz 3, DE-20146, Hamburg, Germany*
[7]*Department of Entomology, National Museum, Cirkusová 1740, Praha 9, Czech Republic*

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This study summarizes available data on karyotypes of water scavenger beetles (Coleoptera: Hydrophiloidea: Hydrophilidae), based on newly acquired data of 23 genera and 64 species. We combine these data with previously published data, which we review. In total, karyotypes are available for 33 genera and 95 species, covering all subfamilies and tribes. Available data indicate that most groups of the Hydrophilidae are diploid and sexually reproducing, with XY (♂) and XX (♀) sex chromosomes; the Y chromosome is always minute and does not recombine with X during meiosis. Exceptions are known in *Anacaena*, with parthenogenetic diploid or triploid populations in some species and sex chromosomes fused with autosomes in others. The diploid number of chromosomes is $2n = 18$ in the subfamilies Acidocerinae, Chaetarthriinae, Enochrinae and Hydrophilinae. Variations are known in species of *Anacaena* and *Berosus* (both usually with $2n = 18$) and in *Hydrochara* and *Hydrophilus* with an increased number of chromosomes ($2n = 30$). The number of chromosomes is increased in the subfamily Cyclominae ($2n = 24–30$) and in all subclades of the subfamily Sphaeridiinae ($2n = 22–32$). We summarize protocols for obtaining chromosome slides used for this study and provide step-by-step guidelines to facilitate future cytogenetic studies.

ADDITIONAL KEYWORDS: chromosome slides – cytogenetics – preparation methods – sex chromosomes.

INTRODUCTION

Figures of mitotic and meiotic nuclei provide us with basic data about the organization of nuclear DNA during cell division (number of chromosomes, their morphology and their interactions during mitosis and meiosis) and how sex is determined (Smith & Virkki, 1978; Schulz-Schaeffer, 1980; Petitpierre, 1996; Appels *et al.*, 1998; Blackmon *et al.*, 2017). Irregularities in chromosome number or morphology may help to recognize hybrids (Nalepa *et al.*, 2017; Traut *et al.*, 2018) or parthenogenetic populations (Lachowska *et al.*, 2008; Milani *et al.*, 2009; Porter & Martin, 2011; Blackmon & Demuth, 2015a). Differences between karyotypes may identify cryptic species (Angus, 1982, 2010; Kobayashi *et al.*, 2000; Angus & Aouad, 2009; Lachowska *et al.*, 2009; Mills & Cook, 2014; Golub *et al.*, 2018). However, karyotype evolution is more dynamic than initially expected; in many cases

*Corresponding author. E-mail: mfikacek@gmail.com
‡née Gray

it results in similar genomes (e.g. those of related species) organized in different karyotypes (Yang *et al.*, 1995; Aniskin *et al.*, 2006; Ferguson-Smith & Trifonov, 2007; Schneider *et al.*, 2010; Bracewell *et al.*, 2019). Some biologists hence consider basic cytogenetics as outdated and karyotype data as not easy to interpret (Petitpierre, 1998; Dobigny *et al.*, 2004; Heng *et al.*, 2013). Modern fluorescent methods capable of testing chromosome homology (Cui *et al.*, 2016), combined with whole-genome sequence data (Doležel *et al.*, 2014; Deakin *et al.*, 2019) and analysed by model-based methods specifically designed to reconstruct karyotype evolution (Dobigny *et al.*, 2004; Freyman & Höhna, 2018), help us to understand these intricate patterns when necessary. However, these methods are dependent on the availability of basic karyotypes, which are starting points for more detailed studies. As yet, basic karyotype data are not available for most insect lineages.

Beetles (Coleoptera) are one of the best-studied groups of insects, with basic cytogenetic data available for nearly 5000 species (Blackmon & Demuth, 2015b, 2020). However, these data do not represent all beetle lineages. We have data on many species of traditionally-studied groups like ground beetles (Carabidae, *c*. 1000 species) and leaf beetles (Chrysomelidae, *c*. 950 species), but limited or no information about many other beetle lineages. We know little about the beetle suborders Myxophaga and Archostemata, with only one and two species karyotyped, respectively (Smith & Virkki, 1978; Mesa & Fontanetti, 1985; Galian & Lawrence, 1993). No data are available for 120 of the 176 beetle families of the suborders Adephaga and Polyphaga (Blackmon & Demuth, 2020). Even in well-studied groups, available data do not cover all phylogenetic lineages, but usually focus on a particular genus or group. For example, 230 of the 1000 karyotyped species of Carabidae belong to a single genus, *Bembidion* Latreille, 1802 (Blackmon & Demuth, 2020). The need for living specimens and basic laboratory equipment moreover biases the sampling towards easy-to-collect species from Europe and North America. In contrast, many tropical or difficult-to-collect taxa have never been examined.

The superfamily Hydrophiloidea contains *c*. 3400 species distributed worldwide (Short & Fikáček, 2011), most of which inhabit aquatic or semi-aquatic habitats (Fikáček *et al.*, 2012b; Short & Fikáček, 2013; Bloom *et al.*, 2014). The superfamily consists of six families, of which Hydrophilidae is most diverse as it concerns number of species (c. 2900), number of genera representing distict morphotypes (c. 180) and habitats the beetles prefer (with c. 1000 species in terrestrial habitats like dung or leaf litter). *Hydrophilus* Geoffroy, 1762 beetles were the first representatives of the Hydrophiloidea in which chromosomes were investigated in a study describing the mitosis and meiosis in the testes (Arnold, 1909). Eight more hydrophilid species were examined later (Asana *et al.*, 1942; Smith, 1953, 1960; Agarawal, 1960a, 1960b); the data were published for eight species; however, except for the number of chromosomes, they are not easy to interpret given the methods used (serial sectioning and squashing, see below). The discovery of the hypotonic inflation and air-drying technique (Crozier, 1968) made the chromosome preparations easier to prepare and interpret. It was adapted and first used for the Hydrophiloidea in a study of species limits in the *Helophorus aquaticus* (Linnaeus, 1758) group (Angus, 1982). Angus later continued in gathering cytogenetic data for other *Helophorus* Fabricius, 1775 species and applied them in studies of species-level systematics. Hence, the family Helophoridae is among the best-studied groups of beetles, with 56 species (nearly one-third of the total number of species) karyotyped until today (Angus, 1982, 1983, 1986, 1989, 1992, 1996, 2015; Angus & Díaz Pazos, 1990; Angus *et al.*, 2005, 2016; Angus & Aouad, 2009; Angus & Toledo, 2010; Angus & Jia, 2020). Other hydrophiloid groups received much less attention. Shaarawi (1989) performed the first study of European Hydrophilidae and other Hydrophiloidea in her unpublished PhD thesis, of which only results concerning the Georissidae, Hydrochidae and Spercheidae (Shaarawi & Angus, 1992), and the hydrophilid genera *Anacaena* C.G. Thomson, 1859, *Berosus* Leach, 1817, *Chaetarthria* Stephens, 1833 and *Laccobius* Erichson, 1837 were published (Shaarawi & Angus, 1991a, 1991b; Angus & Shaarawi, 1997). Further data were added more recently for the hydrophilid genera *Berosus*, *Sphaeridium* Fabricius, 1775 and *Tropisternus* Solier, 1834 (Angus *et al.*, 1994, 2000; Pine *et al.*, 2013).

The present study aims to provide basic karyotype data for all subfamilies and tribes of the family Hydrophilidae, covering as many genera and species as possible. These data should facilitate subsequent analyses of genome evolution in the family (Fikáček *et al.*, in prep.) and provide basic information about variation of chromosome numbers and the chromosome morphology within the tribes, genera and species. The study is based on data of European Hydrophilidae prepared between 1980–2018 by R.B.A, F.S. and H.D. We complement these data with results obtained during the recent field work in New Zealand, Taiwan and the Dominican Republic. We also provide a summary of methods used, to facilitate further studies of chromosomes in the superfamily Hydrophiloidea and other groups of beetles.

Development of beetle karyotype preparation methods

Although *Hydrophilus piceus* (Linnaeus, 1758) is among the earliest beetles to have their chromosomes investigated (Arnold, 1909), these early studies were concerned with the nature of sex determination and numbers of chromosomes. They used serial sectioning and were not able to give information on chromosome morphology. Methods of chromosome preparation were reviewed by Smith (1943), who described smearing as the best method for obtaining chromosomes from the testis and squashing being of more general use. Squashing tends to distort (stretch) chromosomes, particularly near the edges of nuclei. Nevertheless, Smith (1966) produced excellent pictures of *Chilocorus* Leach, 1815 (Coccinellidae) chromosomes and was able to demonstrate natural hybridization between species with adjoining distributions.

The major breakthrough in obtaining preparations of mitotic chromosomes suitable for studying karyotypes came from the use of hypotonic inflation and air-drying techniques. Crozier (1968) used brain ganglia dissected from ant pupae. The ganglia were dissected in buffered insect saline with colchicine to abolish spindle formation at mitosis. Then the cells were inflated by immersion in a 1% solution of trisodium citrate before being fixed in a 3:1 solution of absolute ethanol (or methanol) and glacial acetic acid. Pieces of tissue were placed on a microscope slide, and the cells were dissociated in a drop of 60% acetic acid. The application of a drop of fixative caused the cell suspension to spread as a thin film, which was allowed to dry. Crozier (1968) stained his preparations with acetic-lactic orcein for about 12 h. Angus (1982) made various modifications to this technique: (1) the use of 1% Giemsa stain resulted in good staining after 10 min; (2) replacement of trisodium citrate by a half-isotonic solution of potassium chloride gave a clearer outline to the chromosomes and also helped preserve chromomere banding; and (3) reduction of the acetic acid cell dissociation solution to 45% also improved chromomere banding.

Traditionally, chromosome preparations were made from testes or ovaries of adult beetles, which required beetles of a suitable age for gametogenesis to be taking place. If beetles were too old, only sperm would be present in the testes, and the ovaries would not have any oogonial mitosis. Using embryos in developing eggs was an alternative way. A further breakthrough was the discovery by Angus that the midgut epithelium was an additional source of dividing cells. There was a view among insect cytogeneticists that since adult insects do not grow, their somatic cells would not undergo mitosis. Angus's discovery was made by accident, although mitosis in the midgut of adult beetles, where regenerative cells replace epithelial cells lost after enzyme production, is well known to physiologists (Chapman, 1998; Nardi & Bee, 2012). Angus (1989) illustrated chromosomes obtained from the midgut, and the technique was first described by Shaarawi & Angus (1991b). The final set of methods used for this paper is outlined in the *Material and Methods* below.

MATERIAL AND METHODS

The new karyotypes presented in this study are based on: (1) the earlier work by R.B.A., F.S. and H.D. [European species, *Dactylosternum flavicorne* (Mulsant, 1844) and *Hydrobius pui* Jia, 1995] and (2) on the species from Taiwan and New Zealand obtained during the recent field-work; the slide preparations of most of these specimens were done by D.S., R.B.A. and in some cases by M.F. We intended to present male and female mitotic karyotypes and the figures of meiotic metaphase I, in order to recognize sex chromosomes and reveal their meiotic interactions. Males were not available for some species and hence only female mitotic karyotype was obtained; in such cases sex chromosomes cannot be recognized and are not indicated in karyograms, and only the number and morphology of chromosomes are analysed. In *Noteropagus* d'Orchymont, 1919 and *Protosternum* Sharp, 1890, we only obtained few meiotic figures from the testes which were good enough to estimate the number of chromosomes but did not allow further evaluation. These results are published as well, because both genera are rare and *Protosternum* is the only representative of the tribe Protosternini examined. We present karyograms or meiotic chromosomes for all newly examined species; the complete set of all unedited photographs of mitotic and meiotic nuclei are available in the Zenodo archive under the doi: http://doi.org/10.5281/zenodo.3950844. Voucher specimens are deposited in The Natural History Museum, London (BMNH) and the Department of Entomology, National Museum in Prague (NMPC). Xy_p indicates the small size of the Y chromosome and the position of the X and Y chromosomes during meiosis, which are separated and do not recombine (the so-called 'parachute formation') (Blackmon & Demuth, 2015b).

We used two different methods (the original way with colchicine used by R.B.A., and the adapted way without colchicine used by D.S.) and various tissues for obtaining the karyotypes presented in this study. In order to facilitate the future cytogenetic studies of beetles, we summarize our methods in a form of practical guidelines below.

Method with colchicine

Chemicals needed: (1) Colchicine solution: 0.1% colchicine in insect saline (0.75% NaCl) buffered to pH 6.8 [i.e. mix 0.75 g NaCl + 100 mL working Sörensen's buffer solution (see below under *Staining* for the recipe) and add 0.1 g colchicine powder (be careful: colchicine is poisonous and should not be inhaled as a powder or ingested as a solution)]; (2) half-isotonic KCl: 4.8 g KCl + 1000 mL working Sörensen's buffer solution; (3) ethanol fixative: three parts absolute ethanol + one part glacial acetic acid; (4) 45% acetic acid in distilled water. The fixative needs to be mixed just before slide preparation.

General protocol: (1) Expose the living specimen to the colchicine solution for *c*. 12–15 min, either by injection or by partially detaching the abdomen; (2) transfer the specimen to half-isotonic KCl, dissect the internal organs, incubate for 12 min; (3) move the tissue to the ethanol fixative and leave for 30–60 min, change the fixative twice during fixation; (4) transfer the tissue onto a dry clean microscopic slide and immediately apply a small drop of 45% acetic acid to disaggregate the tissue (tear the tissue apart with fine insect pins in case it does not disaggregate enough); (5) when the tissue disintegrates, apply a small drop of the fixative causing the liquid to spread as a thin film over the slide, move the drop across the slide by tilting it until it dries up. Do not add too much acetic acid or fixative as this results in the cells being carried to the edges of the slide.

This method is used routinely by R.B.A. and was also described by Angus (2006) and Angus & Jia (2020).

Method without colchicine

Chemicals needed: (1) Hypotonic solution 0.075M KCl in distilled water (5.6 g KCl + 1000 mL distilled water); (2) methanol fixative: three parts methanol + one part glacial acetic acid; (3) 60% acetic acid in distilled water. It is best to mix all chemicals shortly before the laboratory work; when this is not possible, at least the fixative needs to be mixed just before the slide preparation.

General protocol: (1) Dissect the living specimen in hypotonic KCl solution, clean the midgut or gonads of other tissues, wait 25 min; (2) transfer the tissue to the fixative, wait for 5 min and transfer the tissue to fresh fixative and wait for another 10 min. (3) Transfer the fixed tissue onto a clean dry slide (ideally SuperFrost); (4) add 1–2 drops 60% acetic acid to disintegrate the tissue, suspend it mechanically with fine tungsten wire, remove undissociated clusters; (5) put the slide with suspension on a warm plate (45 °C) and move the drop around the slide with the tungsten wire until it dries up totally.

This method is used routinely by D.S. for the Heteroptera (Sadílek *et al.*, 2016); it works well for hydrophiloid beetles as tested in this study.

Tissues used for chromosome slides

We used live specimens for all chromosome preparations. In Europe, we brought live specimens in suitable containers allowing for air circulation and containing some humid substrate (usually moss or moist filter paper) to the laboratory where they were dissected. Specimens should be dissected shortly after collecting or allowed to feed on a suitable substrate for a day before dissection in case the midgut is intended to be used for mitotic karyotypes. Specimens collected during expeditions outside Europe were dissected directly at the field accommodation under a small portable binocular microscope and slides were prepared in the usual way described above. Necessary liquid chemicals were obtained in the respective country from local colleagues, since they are flammable or corrosive and cannot be transported by plane. A small heating plate was carried in checked luggage. Final dry slides were transported as carry-on luggage or carefully wrapped in multiple layers of cloth in checked luggage. The technique used (spreading) does not allow for the use of fixed specimens (for details, see Sadílek *et al.*, 2016); however, it is simple enough to be performed directly in the field and hence to obtain karyotypes from exotic species.

Embryos: Under laboratory conditions, embryos are at a suitable stage of development 2–3 days after the eggs have been laid. Figure 1A shows the egg of *Helophorus* slightly after the right stage as the segmentation of the embryo is already clear. To prepare chromosomes from developing embryos, some eggs need to be removed from an opened egg case to a watch glass containing colchicine solution. The eggshells are pierced with a sliver of glass broken from a coverslip and left for about 15 min. Then the liquid is pipetted off and replaced by half-isotonic KCl. The eggs are squeezed between the tips of fine forceps so that the contents are extruded. After 12 min almost all the liquid is pipetted off and replaced by the ethanol fixative gently pipetted on to it; the fixative is changed twice, and the watch glass is covered and left for 30–60 min. Pieces of tissue are then gently pipetted onto microscope slides, and before they dry out a small drop of 45% acetic acid is applied with a hypodermic

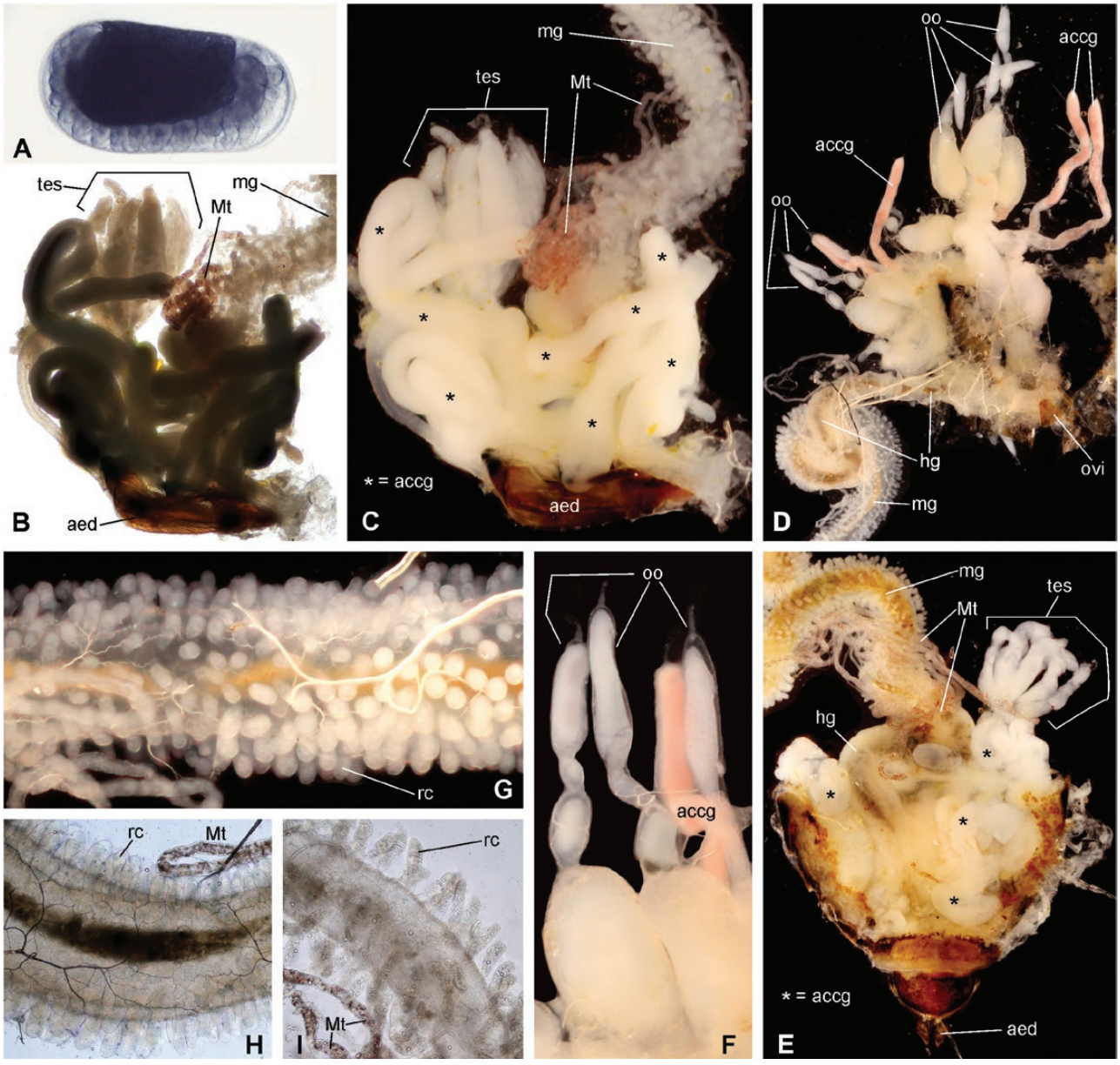

Figure 1. Tissues of hydrophiloid beetles used for chromosome preparations (as dissected, without any additional treatment, (B–E) in dorsal view; (A) *Helophorus grandis*; (B, C, I) *Laccobius bipunctatus*; (D–H) *Coelostoma orbiculare*). A, egg with a developing embryo. B–C, internal organs of the same male specimen in translucent light (B) and on black background (C). D, internal organs of a female with digestive system pulled aside. E, internal organs of male specimen. F, details of ovarioles and associated accessory glands. G–I, detail of midgut structure on black background (G) and in translucent light (H, I). Abbreviations: accg, accessory glands; aed, aedeagus; hg, hindgut; mg, midgut; Mt, Malpighian tubes; oo, ovarioles; ovi, ovipositor; rc, regeneration crypts; tes, testes. Not to scale.

syringe to minimize the amount dropped. Once the tissue disaggregates, a drop of fixative is applied.

Internal organs of adult beetles: Follow the procedure described above under the colchicine or non-colchicine methods. Dissect the specimen once in KCl solution using fine hard forceps and fine stainless steel insect pins. In contrast to the processing of eggs, dissected tissues are transferred to a new watch glass with a respective chemical or to a dry microscopic slide by fine forceps. The pieces of tissue in 45% or 60% acetic acid need to be torn apart with fine pins to disintegrate properly.

Midgut: The midgut is easy to recognize from other parts of the digestive system and other internal organs according to the finger-like projections (regeneration crypts) on its surface (Fig. 1B–E, G–I). Stem cells are dividing quickly in the distal part of these projections and mitotic chromosomes may be found (Nardi & Bee, 2012). For chromosome preparation, the complete midgut is used. Cell division in regeneration crypts is abundant only in actively feeding specimens, hence avoid the use of inactive specimens at the beginning or at the end of the season. Ideally, food particles should be seen in the dissected midgut indicating that the specimen was feeding within 24 h before being dissected. The end of the midgut is indicated by bundles of Malpighian tubes (usually yellowish or pinkish in colour), which open to the midgut at its end (Fig. 1B, C, E); only parts anterior to the attachment of the Malpighian tubes should be used. The sex of the specimen needs to be noted down.

Testes: Testes of the Hydrophiloidea consist of a bunch of finger-like follicles opening to the vas deferens at a single place or a few closely adjacent places (Fig. 1B, C, E); they are of the fasciculate or cluster type (Matsuda, 1976). They should not be confused with the accessory glands that are usually massive and hence more apparent in the Hydrophiloidea (e.g. Fig. 1C, E) and which may be pseudosegmented (e.g. in *Anacaena*). In young or old specimens, the testes may be difficult to locate among the accessory glands. In young specimens, the testes only contain spermatogonial mitosis (i.e. no meiosis). In old specimens, the testes and vas deferens are filled with bundles of spermatozoa which give them a mottled appearance, and the vas deferens becomes distended; at this stage no cell division is present. Other groups of beetles have testes of slightly different morphology: with a single follicle which may be coiled into a "wool ball" in the Adephaga, with multiple spherical follicles in scarabs, chrysomelids and curculionids (Virkii, 1957; Suzuki, 1988; Nasserzadeh *et al.*, 2005; Will *et al.*, 2005; Wu *et al.*, 2017). The morphology in a particular group should be checked before dissection in order to take the correct tissue. If the testes cannot be located when rare or hard-to-collect specimens are dissected, we recommend using the whole internal male genital system which can be separated in sections of similar morphology and put on several slides. Spermatogenesis is known to occur in pre-adult stages in some holometabolous insects (Economopoulos & Gordon, 1971; Chapman, 1998; Kerkut & Gilbert, 2013) and hence testes of adults may not be a good source of mitotic or meiotic cells in all beetle groups.

Ovaries: In hydrophiloid beetles, the ovaries usually develop as bundles of finger-like apically tapering projections (ovarioles) easy to recognize once the digestive system is removed (Fig. 1D, F). In some groups they may be intermixed with accessory glands, which do not taper apically or may be shorter and more robust (De Marzo, 2008). Whole ovaries are used for chromosome slides but they only show mitotic nuclei as meiosis is usually triggered only in fertilized eggs (Chapman, 1998). Despite our trials to obtain chromosomes from the ovaries, we never succeeded with the Hydrophilidae or *Helophorus*. It may indicate that cell divisions are infrequent in the ovaries of adult hydrophiloid beetles. Mitotic chromosomes were infrequently gained from the ovaries of carabid and dytiscid beetles (Rozek, 1985, 1988; Angus *et al.*, 2013) but are routinely gained from ovaries of subadult larvae or adults of Heteroptera (Sadílek *et al.*, 2013).

Staining

Chemicals

(1) Sörensen's buffer working solution: prepare two master solutions: 1/15 M $Na_2HPO_4.12H_2O$ (23.88 g $Na_2HPO_4.12H_2O$ + 1000 mL distilled water) and 1/15 M KH_2PO_4 (9.07 g KH_2PO_4 + 1000 mL distilled water); mix the working solution as 50 mL $Na_2HPO_4.12H_2O$ master solution + 50 mL KH_2PO_4 master solution + 900 mL distilled water (alternative way used by D.S.: mix 4.75 g $Na_2HPO_4.12H_2O$ + 4.54g KH_2PO_4 in 1000 mL distilled water, adjust pH to 6.8 by adding few drops of HCl or NaOH).

(2) Giemsa solution: A 1–2% solution in Sörensen's buffer (i.e. 1–2 mL Giemsa + 100 mL Sörensen's buffer working solution) is used by R.B.A.; 5% solution in Sörensen's buffer (i.e. 5 mL Giemsa + 100 mL Sörensen's buffer working solution) is used by D.S.

(3) $Ba(OH)_2$ solution: saturated solution (5 g $Ba(OH)_2$ in 500 mL distilled water mixed at 65 °C, then heated to 95 °C and filtered; alternative way: put crystalline $Ba(OH)_2$ in a bottle, fill with distilled water, stopper and shake, if all the crystals have gone into a cloudy solution, add more; the saturated solution at room temperature should have crystals of $Ba(OH)_2$ and precipitated $BaCO_3$ at the bottom).

(4) 2 × SSC (salt-sodium citrate): 1.75 g NaCl + 0.88 g trisodium citrate + 100 mL distilled H_2O.

Giemsa staining: After at least one hour and preferably the day after preparation, the slides are stained by immersion in Giemsa solution (10 min in 1–2% solution was used by R.B.A., 30 min in 5% solution by D.S., the time needs to be optimized for each group). The next day, to be sure they are completely dry, the slides may be examined under low magnification and photographed under oil immersion.

C-banding: In slides previously examined with immersion oil, the oil and Giemsa stain need to be removed by two changes of xylene + one change of absolute ethanol (or one change of xylene + one change of medical benzine + one change of methanol fixative), then slides are allowed to dry. C-banding is produced by immersing the slides in $Ba(OH)_2$ solution for about 5 min at room temperature (about 23 °C). The slides are then rinsed in three changes of Sörensen's buffer, then incubated for 1 h in 2 × SSC at 60 °C. They are then rinsed in three changes of Sörensen's buffer and stained for 10 min in 1% Giemsa. This procedure can be repeated if the C-banding is considered to be insufficient.

Observation and photography

The hydrophiloid chromosomes are relatively small, ranging from *c*. 1–15 µm in length (with minute Y chromosome even smaller). The initial observation after the Giemsa staining needs to be hence done with magnification of *c*. 20–40× with properly set light and light condenser [close the condenser partly and keep the light fairly low to be comfortable]. Once useful figures are located, they need to be photographed with 100× objective under oil-immersion. Use of a monochromatic green interference filter enhances the contrast. Before the staining, the slides may be quickly screened for presence of chromosome figures under phase contrast at 10–20×. Karyogram assembly is done by pairing up chromosomes based on size and shape and any other distinguishing features. When photographs of the same nucleus without treatment and C-banded are available, the banded and unbanded preparations should be treated together. Karyograms are assembled from single nuclei. If a chromosome is missing from the chosen nucleus (in rare species with limited number of nuclei available), its position should be indicated by a sign (black square, for example).

RESULTS

SUBFAMILY HYDROPHILINAE

TRIBE AMPHIOPINI

GENUS AMPHIOPS ERICHSON, 1843

(FIG. 2A–E)

Material examined: Amphiops mater Sharp, 1873: 2 females (BMNH); 1 female (NMPC): Taiwan, Beipu, 24.707467°N 121.060870°E, 12.ix.2018, H.-C. Liu lgt.

Karyotype: $2n = 18$ (♀). The largest pair of chromosomes is submetacentric, the remaining pairs are metacentric. C-banding shows clear centromeric C-bands and terminal C-bands (heterozygous) on pairs 2 and 9.

TRIBE BEROSINI

GENUS *BEROSUS* LEACH, 1817

Comments on karyotypes: Shaarawi & Angus (1991a) published the karyotype data of three species of the subgenus *Berosus* [*Berosus affinis* Brullé, 1835, *Berosus luridus* (Linnaeus, 1761) and *Berosus signaticollis* (Charpentier, 1825)] and two species of the subgenus *Enoplurus* (*Berosus bispina* Reiche & Saulcy, 1856 and *Berosus fulvus* Kuwert, 1888) and Angus *et al.* (1994) added *Berosus* (s.s.) *hispanicus* Küster, 1847. The karyotype has $2n = 16 + Xy_p$ (♂), in all species except *Berosus signaticollis* which has an extra pair of autosomes $2n = 18 + Xy_p$ (♂). The karyotype of *Berosus affinis* may have an additional pair of B-chromosomes. The parachute configuration is shown by meiotic preparations of *Berosus signaticollis*. C-banding was not attempted; however, the chromosomes appear elongate and with small centromeric constrictions suggesting that the centromeric C-bands are small. Agarawal (1960b) presents data on mitosis and meiosis of *Berosus* (*Enoplurus*) *indicus* (Motschulsky, 1861), providing the meioformula $8 + Xy_p$ which agrees with newer data; the species identification by Agarawal (1960b) may be easily incorrect since no reliable identification key for Asian *Berosus* existed at that time.

TRIBE LACCOBIINI

GENUS *LACCOBIUS* ERICHSON, 1837

(FIG. 2F–L)

Material examined: Laccobius (*Compsolaccobius*) *decorus* (Gyllenhal, 1827). 2 males. (BMNH): Sweden, Öland Island, R. Angus lgt.

Karyotype: $2n = 16 + Xy_p$ (♂). Centromeric C-bands strong. All autosomes, and X chromosome, metacentric. Autosomes similarly sized, X chromosome the largest in the nucleus.

Comments on karyotypes of Laccobius: Angus & Shaarawi (1997) published the karyotype data of two species of the subgenus *Laccobius* [*Laccobius colon* (Stephens, 1829) and *Laccobius minutus* (Linnaeus, 1758)] and four species of the subgenus *Dimorpholaccobius* [*Laccobius bipunctatus* (Fabricius, 1775), *Laccobius sinuatus* Motschulsky, 1849, *Laccobius striatulus* (Fabricius, 1801) and *Laccobius*

ytenensis Sharp, 1910]. *Laccobius* karyotypes all show $2n = 16 + Xy_p$ (♂). All autosomes and the X chromosome have strong centromeric C-bands, which are particularly strong in *Laccobius striatulus* and *Laccobius sinuatus*.

Genus *Paracymus* Thomson, 1867
(Fig. 3A–H)

Material examined: Paracymus aeneus (Germar, 1824) : 2 spec. (BMNH): United Kingdom, England, Isle of Wight, R. Angus lgt.

Paracymus scutellaris (Rosenhauer, 1856): 2 spec. (BMNH): United Kingdom, England, Hampshire, New Forest, R. Angus lgt.

Karyotype: $2n = 16 + Xy_p$ (♂). No C-banding was attempted. Autosome 1 is distinctly longer than the others and X is the shortest (apart from y) in both species.

Genus *Tormus* Sharp, 1884
(Fig. 3I–M)

Material examined: Tormus helmsi Sharp, 1884: 1 female (NMPC): New Zealand: Buller (BR) Tobins Bridge at Hwy. 7 (11.6 km NWW of Springs Junction), 42.29202°S 172.05333°E, 527 m, 3.xii.2016, M. Fikáček & M. Seidel lgt. (MM51).

Tormus posticalis (Broun, 1917): 1 male (NMPC): New Zealand, Westland (WD), Stream at Jackson River Rd., Red Hill area, 44°07.41'S 168°32.87'E, 150 m, 7.xii.2017, sifting dry moss, M. Fikáček, D. Sadílek & V. Sýkora lgt. (2017-NZ73).

Karyotype: $2n = 16 + Xy_p$ (♂). All autosomes and the X chromosome are metacentric. Autosomal pairs 1 and 2 are distinctly longer than the others which decrease in length gradually.

Tribe Hydrobiusini
Genus *Hydrobius* Leach, 1815
(Fig. 4A–J)

Material examined: Hydrobius fuscipes (Linnaeus, 1758): 2 spec. (BMNH): United Kingdom, England, Sheppey Island, R. Angus lgt.; 1 spec. (BMNH): Spain: Menorca, G. Foster lgt. [specimen barcoded by J. Bergsten and confirmed to belong to *Hydrobius fuscipes sensu* Fossen *et al.* (2016)].

Hydrobius arcticus Kuwert, 1890: 1 spec. (BMNH): Sweden, Abisko National Park, G. N. Foster & A. N. Nilsson lgt. [specimens from this locality barcoded by J. Bergsten and confirmed to belong to *H. arcticus sensu* Fossen *et al.* (2016)].

Hydrobius rottenbergii Gerhardt, 1872: 1 spec. (BMNH): Sweden, Angermanland, Nordmaling, Järnäsklubb 63.4314°N, 19.6597°E, rockpools by the sea, 13.x.2017, A.N. Nilsson lgt. [Material from this locality barcoded by J. Bergsten and confirmed to belong to *H. rottenbergii sensu* Fossen *et al.* (2016)].

Hydrobius subrotundus Stephens, 1829: 3 spec. (BMNH): England, Westmorland, swamp near Borderside, 54.305°N 2.876°W. 34 m a.s.l., 18.iii.2016, G. Foster lgt.; 2 spec. (BMNH): France, Aubrac, R. Angus lgt.; 2 spec. (BMNH): Scotland, Kype Muir, S. Routledge lgt. [one specimen barcoded by J. Bergsten and confirmed to belong to *H. subrotundus sensu* Fossen *et al.* (2016)].

Hydrobius pui Jia, 1995: 9 spec. (BMNH): China, Sichuan, Maba Prefecture, Songpan County, pools beside Chuanzhusi-Huanglong, road, by Mt Baoxueding, 3650 m a.s.l., 32°43'37.19"N 103°40'0.7"E, 8.vii.2016, R. Angus & F-L Jia lgt. [These specimens were sequenced by J. Bergsten and indicated to belong to the same barcoding clade as specimens from Kangding County, Xinduqiao, Gaba, Liqi village, 29°56'35.80"N 101°35'21.46"E, 3359 m a.s.l., 28.vi.2016, R.B. Angus, F-L. Jia, Z-Q. Li & K. Chen lgt. which were not karyotyped. Type locality of *H. pui* is Yushu in southern Qinghai, but the fact that our material from two widely separated localities belongs to the same DNA clade gives confidence that all these specimens are conspecific with *H. pui*.]

Karyotype: $2n = 16 + Xy$ (♂). All species with chromosomes 1 and 2 metacentric, 3–7 submetacentric, and 8 and X subacrocentric, X the smallest apart from dot-like y. In *H. fuscipes*, the English material (Fig. 4A–B) has a centromeric C-band on chromosome 8, Menorcan material (Fig. 4C) has additional bands on chromosomes 5 and 6. *Hydrobius subrotundus* (Fig. 4F–H) chromosomes have the size and form as *H. fuscipes* but often with apical satellite C-bands on chromosomes 3–7. *Hydrobius arcticus* (Fig. 4D) and *H. rottenbergii* (Fig. 4E) are characterized by weak C-bands on the X chromosome. *Hydrobius pui* (Fig. 4I) bears weak centromeric C-bands.

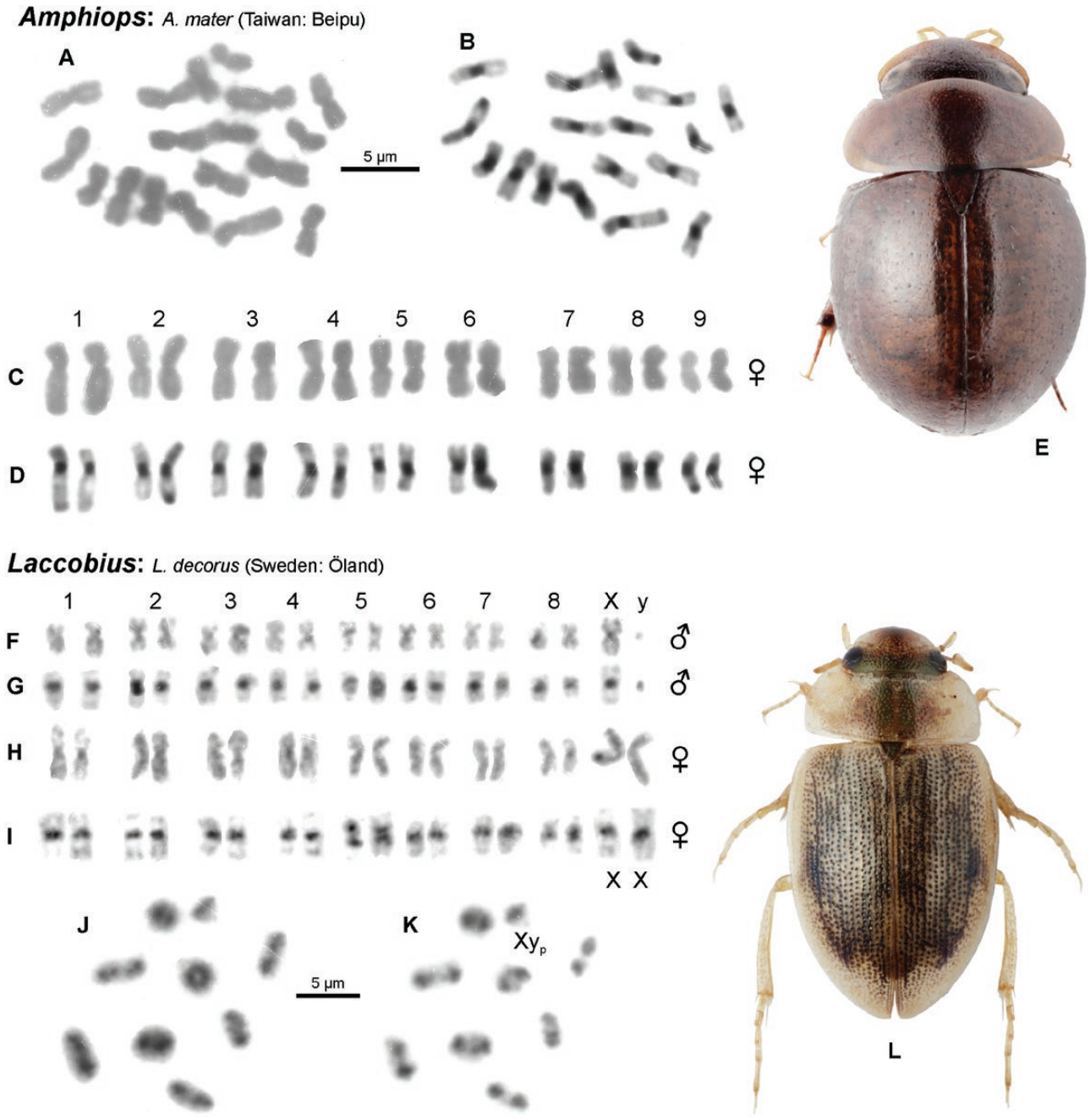

Figure 2. Karyotypes of the Amphiopini and Laccobiini. A–E, *Amphiops mater*, mitotic methaphase from midgut. F–L, *Laccobius decorus,* mitotic metaphase from midgut. A, C, F, H, J, without treatment. B, D, G, I, K, C-banded. Habitus figures: (E) *Amphiops mater*; (L) *Laccobius decorus*.

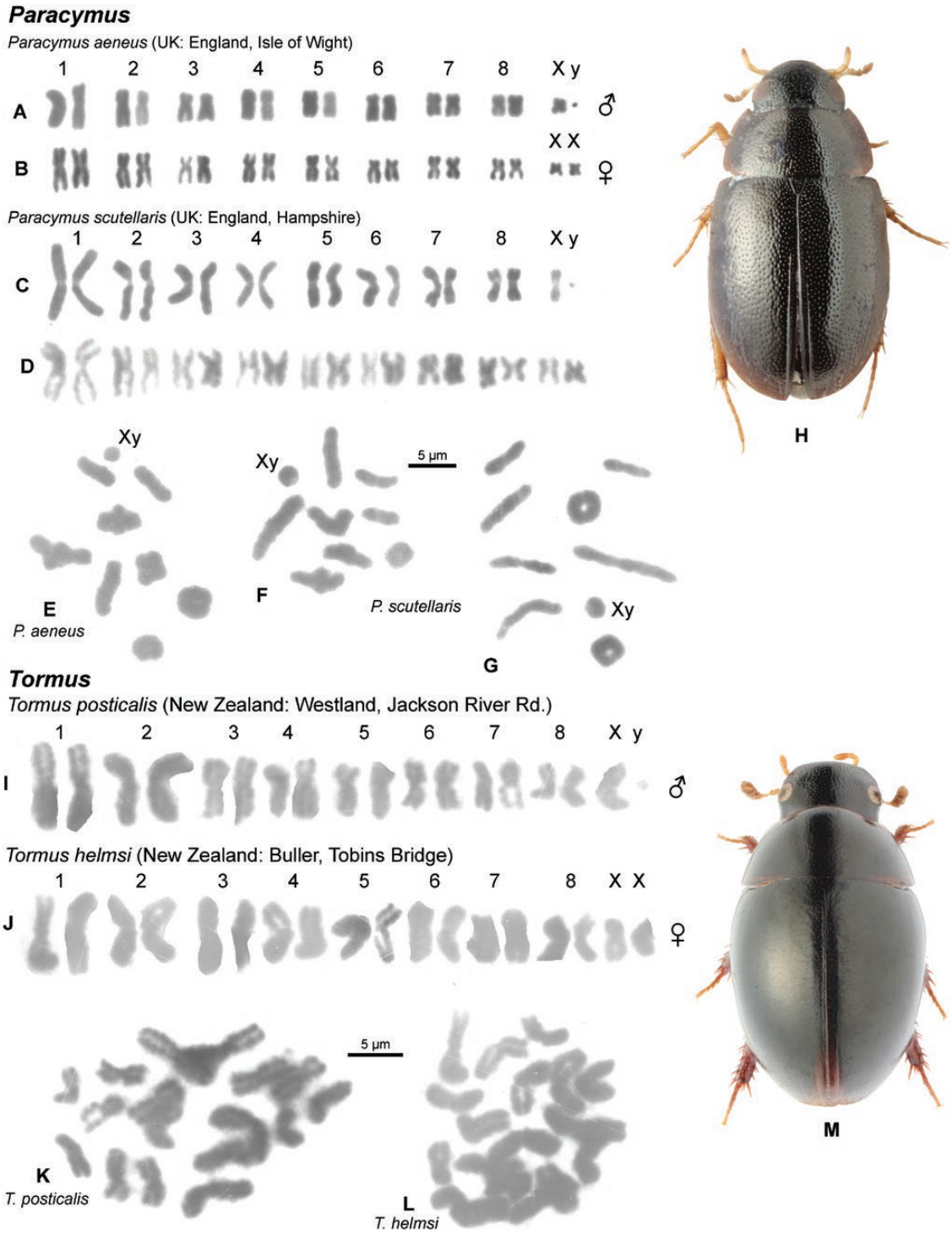

Figure 3. Karyotypes of the Laccobiini, without treatment. A–H, *Paracymus*: (A–B) *Paracymus aeneus*, mitotic metaphase from midgut; (C–D) *Paracymus scutellaris* (C, spermatogonial mitosis, metaphase; D, mitotic metaphase from midgut.); (E–G) meiotic metaphase I from testes (E, *Paracymus aeneus*; F–G, *Paracymus scutellaris*). I–M, *Tormus*, mitotic metaphase from midgut: (I, K) *Tormus posticalis*; (J, L) *Tormus helmsi*. Habitus figures: (G) *Paracymus scutellaris*; (M) *Tormus helmsi*, from Fikáček *et al.* (2013).

Note: Smith (1960) mentions $2n = 18$ for *H. fuscipes* (sensu lato) without any additional information. This agrees with the data presented here.

Genus *Limnohydrobius* Reitter, 1909
(Fig. 5A–E)

Material examined: Limnohydrobius convexus (Brullé, 1835): 2 spec. (BMNH): France, Corsica, R. Angus lgt.; 2 spec. (BMNH): Spain, Menorca, G. Foster lgt. (BMNH).

Karyotype: $2n = 16 + Xy$(♂). Chromosomes 1,3–5,8 and X showing weak centromeric C-bands. Chromosomes 1–6 metacentric, 7, 8 and X submetacentric with X large, about as long as chromosome 5.

Note: Limnohydrobius was recently separated from *Hydrobius* based on DNA-based phylogenetic data (Short *et al.*, 2017); previously *Limnohydrobius* species were classified under the latter genus (e.g. Hansen, 1999).

Genus *Limnoxenus* Motschulsky, 1853
(Fig. 5F–K)

Material examined: Limnoxenus niger (Gmelin, 1790): 2 spec. (BMNH): France, Indre, Brenne, R. Angus lgt.; 1 spec. (BMNH): Greece, Corfu, R. Angus lgt. (BMNH).

Karyotype: $2n = 16 + Xy_p$ (♂). All chromosomes, except the dot-like y, with strong centromeric C-bands. Chromosomes 1, 3, 4 and 7 metacentric, 2 and X subacrocentric, 5–7 acrocentric.

Tribe Hydrophilini
Genus *Hydrochara* Berthold, 1827
(Fig. 6D–H, L)

Material examined: Hydrochara caraboides (Linnaeus, 1758): 2 spec. (BMNH): Greece, Corfu, R. Angus lgt.; 1 spec. (BMNH): Germany, Hamburg, R. Angus lgt. (BMNH).

Hydrochara flavipes (Boheman, 1851): 2 spec. (BMNH): Spain, Cáceres, Abadia, 12.v.1990, R. Angus lgt. (BMNH).

Karyotype: $2n = 28 + Xy$ (♂). The chromosomes showing a gradual decrease in length, the longer chromosomes about 8 µm long, the shorter about 3µm. Centromeric C-bands small but well-developed.

Genus *Hydrophilus* Geoffroy, 1762
(Fig. 6A–C, M)

Material examined: Hydrophilus piceus: 2 spec. (BMNH): Greece, Corfu, R. Angus lgt.

Hydrophilus pistaceus Castelnau, 1840: 1 spec. (BMNH): Spain, Caceres, Abadia, R. Angus lgt.

Karyotype: $2n = 28 + Xy_p$ (♂). The chromosomes are short. C-banding shows all the autosomes with heavy centromeric C-bands accounting for most of their length.

Comments on karyotypes: Mitosis and meiosis were studied in four species of *Hydrophilus* [*Hydrophilus acuminatus* Motschulsky, 1853, *Hydrophilus indicus* (Bedel, 1891), *Hydrophilus piceus* and *Hydrophilus triangularis* Say, 1823] by previous authors (Arnold, 1909; Asana *et al.*, 1942; Smith, 1953; Agarawal, 1960a), all revealing the karyotype $2n = 28 + Xy_p$ (♂), which is in agreement with our findings.

Genus *Sternolophus* Solier, 1834
(Fig. 6I–K)

Material examined: Sternolophus solieri Castelnau, 1840: 1 male (BMNH): Egypt, surroundings of Cairo, 10th Ramadan, R. Angus lgt.

Karyotype: $2n = 16 + Xy$ (♂). Chromosomes 1–7 metacentric, 8 subacrocentric, with an even decrease in length along the karyotype. X chromosome is metacentric, as large as chromosome 1.
Note: Agarwal (1960a) provides a meioformula $n = 8 + Xy_p$ (♂) for *Sternolophus rufipes* (Fabricius, 1792), which agrees with the data presented here.

Genus *Tropisternus* Solier, 1834

Comments on karyotypes: The meioformula of *Tropisternus lateralis* (Fabricius, 1775) was published by Smith (1953, 1960), the karyotype and meiosis of *Tropisternus mutatus* d'Orchymont, 1921 was studied by Pine *et al.* (2013). Published data indicate that *Tropisternus*, like *Sternolophus*, has $2n = 16 + Xy_p$ (♂).

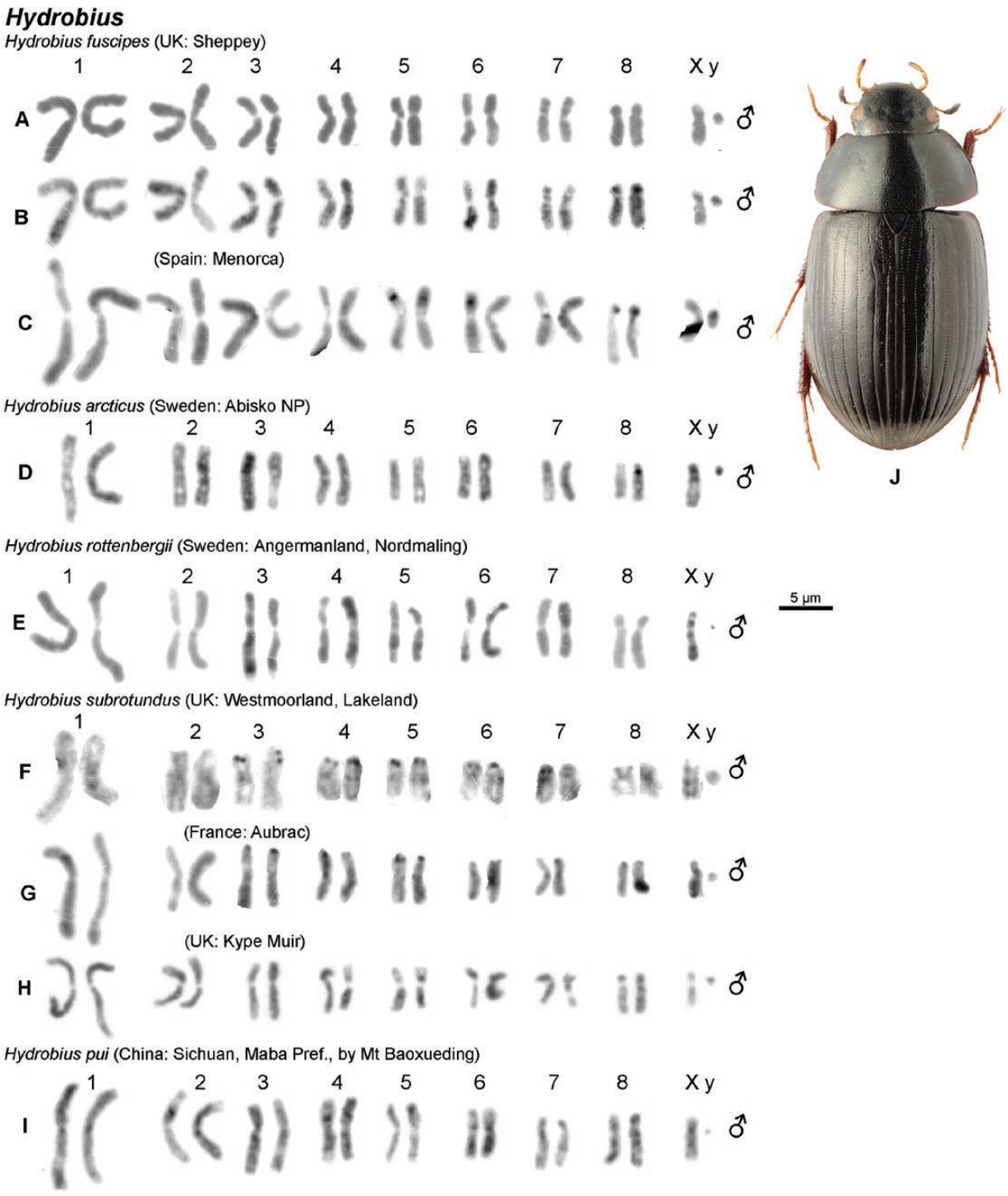

Figure 4. Mitotic karyotypes of *Hydrobius*. A–C, *Hydrobius fuscipes*, testes. D, *Hydrobius arcticus*, midgut. E, *Hydrobius rottenbergii*, testes. F–H, *Hydrobius subrotundus* (F, midgut; G–H, testes). I, *Hydrobius pui*, testes. A, D–I, without treatment. B, C, C-banded. J, habitus of *Hydrobius fuscipes*.

Figure 5. Karyotypes of the Hydrobiusini. A–D, *Limnohydrobius convexus*: (A–B) mitosis, midgut; (C–D): testis, prometaphase. F–J, *Limnoxenus niger*: (F–H) midgut; (I–J) meiotic metaphase I, testes. A–C, E–F, I, without treatment. D, G, H, J, C-banded. Habitus figures: (E) *Limnohydrobius convexus*; (K) *Limnoxenus niger*.

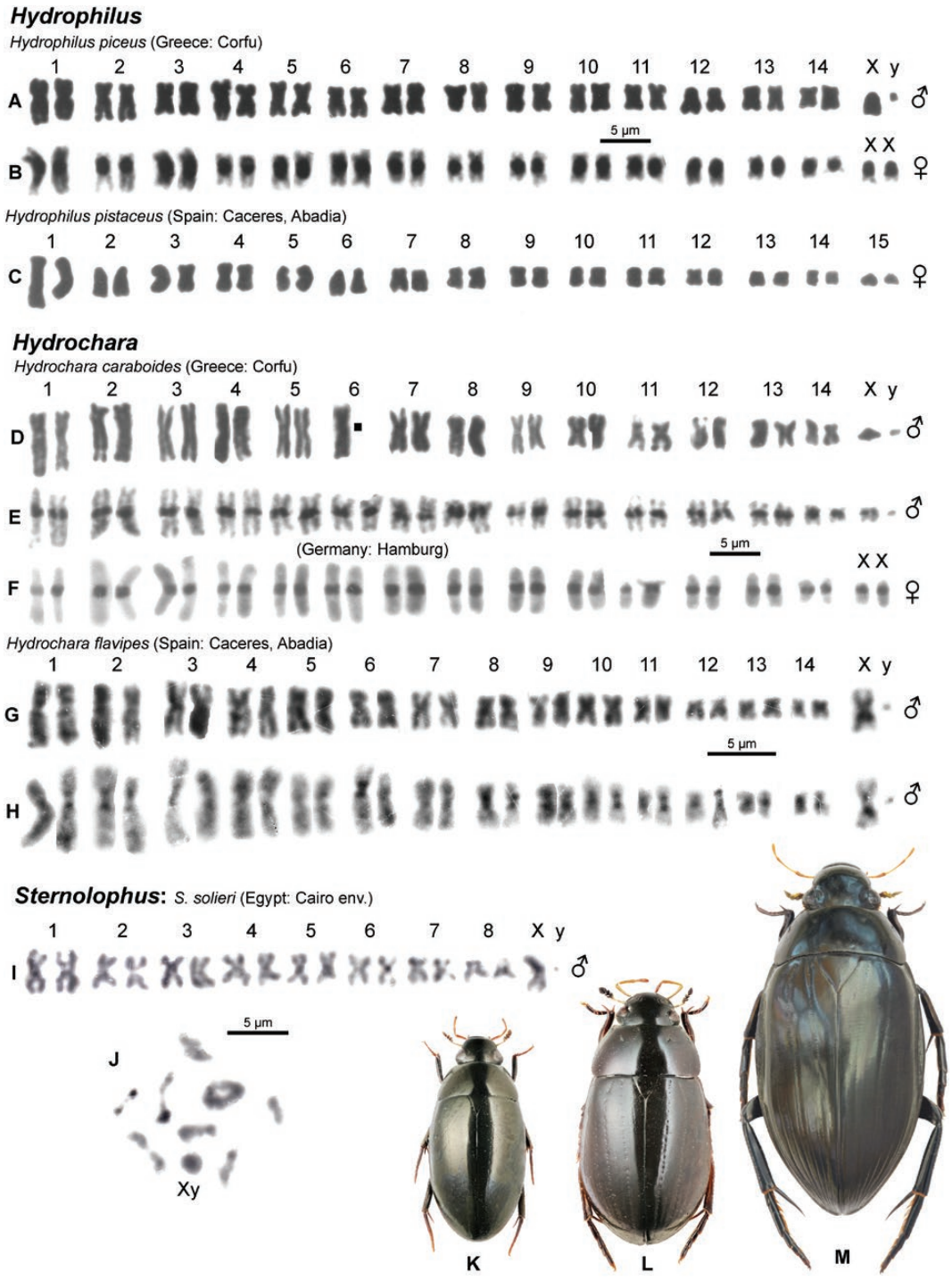

Figure 6. Karyotypes of the Hydrophilini. A–C, *Hydrophilus*, mitotic karyotype from embryo (A–B, *Hydrophilus piceus*; C, *Hydrophilus pistaceus*). D–H, *Hydrochara*: (D–F) *Hydrochara caraboides*, mitotic karyotype, embryo; (G–H) *Hydrochara flavipes*, mitotic karyotype, midgut. I–J, *Sternolophus solieri* (I, male mitotic karyotype; J, meiotic first metaphase from testes). A, C, D, G, I, without treatment. B, E, F, H, C-banded. Habitus figures: (K) *Sternolophus solieri*; (L) *Hydrochara caraboides*; (M) *Hydrophilus piceus*, from Short & Fikáček (2013).

Subfamily Chaetarthriinae

Tribe Anacaenini

Genus Anacaena Thomson, 1859

(Fig. 7A–D)

Material examined: Anacaena gaetanae Bameul, 2001: 1 female (BMNH): France, Corsica, Corse du Sud, R. Stabiacciu, Porto Veccio, 12.vii.2009, R.B. & E.M. Angus lgt. (BMNH).

Anacaena lutescens (Stephens, 1829): 3 female specs. (BMNH): Germany, Hamburg district, 16.iv.1988, R. Angus lgt.; 1 spec. (BMNH): United Kingdom, Norfolk, R. Angus lgt.

Karyotype: The karyotype of *A. gaetanae* (Fig. 7A) is similar to that of bisexual *A. lutescens* ($2n = 16 + Xy_p$ (♂)) but the chromosome taken to be the X is noticeably larger.

Comments on karyotypes: The karyotypes are discussed in detail by Shaarawi & Angus (1991a). Parthenogenetic *A. lutescens* is heterozygous for loss of a small apical section of chromosome 8, distal to a secondary constriction (Fig. 7C). *Anacaena globulus* (Paykull, 1798) has a fusion of two chromosomes (autosomes) to give a reduction from eight to seven pairs ($2n = 14 + Xy_p$). *Anacaena rufipes* (Guillebeau, 1896) has two further fusions, one involving the X chromosome, to give five pairs of autosomes and neo-XY sex chromosomes ($2n = 10 +$ neo-XY). Shaarawi & Angus (1991a) suggested that the original small Y chromosome had also fused with the neo-Y chromosome, but it is also possible that it has simply been lost. Triploidy ($3n = 27$) was found in two widely separated populations of *A. lutescens*, from the United Kingdom (Armathwaite, Cumbria) and the Netherlands (Doetinchem, Gelderland). In both cases diploid females were also present. In the triploids there was one pair of intact chromosome 8 and one extra chromosome 8 with the deletion. In the Armathwaite population, one of three replicates of chromosome 8 has a pericentric inversion and is acrocentric.

Genus *Horelophus* d'Orchymont, 1913

(Fig. 7E–K)

Material examined: Horelophus walkeri d'Orchymont, 1913: 2 males (NMPC): New Zealand, Nelson (NN), Kahurangi Nat. Park, AppleTree Flat at Cobb Dam Road, 200 m, 41.0744°S 172.75761°E, 12.xii.2016, hygropetric habitat: exposed rocks with thin film of water, moss and algae, M. Fikáček & M. Seidel lgt. (MM73). 1 male (NMPC): New Zealand: Marlborough, Pelorus Bridge Scenic Reserve, first waterfall at Tawa Walk, 41.30526°S 173.56739°E, 55 m, floating moss from small waterfall in *Nothofagus* Blume forest, 11.xii.2016, M. Fikáček & M. Seidel lgt. (MM69b).

Karyotype: $2n = 16 + Xy$ (♂). *Horelophus* chromosomes seems to be acrocentric to subacrocentric and decrease in size along the karyotype. X chromosome is about as long as chromosomes 6–8.

Tribe Chaetarthriini

Genus *Chaetarthria* Stephens, 1835

(Fig. 7L–M)

Material examined: Chaetarthria simillima Vorst & Cuppen, 2003: 1 male (BMNH): United Kingdom: England, Oxfordshire, Cothill, R. Angus & F. Shaarawi lgt.

Karyotype: $2n = 16 + Xy_p$ (♂). There are distinct steps in the size decreases between chromosomes 1 and 2, 2 and 3, and 3 and 4, then a gradual decrease between chromosomes 4 and 6, a slightly larger one between 6 and 7 and a sharp decrease between 7 and 8. The X chromosome is about the same length as autosomes 4–6 and the y is dot like. Chromosomes 1–6 and the X chromosome are metacentric, chromosomes 7 and 8 are subacrocentric.

Note: The karyotype was published by Angus & Shaarawi (1997) under the name *Chaetarthria seminulum* (Herbst, 1797). It has been slightly rearranged and improved and it republished here.

Subfamily Enochrinae

Genus *Cymbiodyta* Bedel, 1881

(Fig. 8A, I)

Material examined: Cymbiodyta marginella (Fabricius, 1792): 1 spec. (BMNH): Greece, Corfu, R. Angus lgt.

Karyotype: $2n = 16 + Xy_p$ (♂). Eight pairs of metacentric or submetacentric autosomes showing a gradual decrease in size, pair 8 is about half the

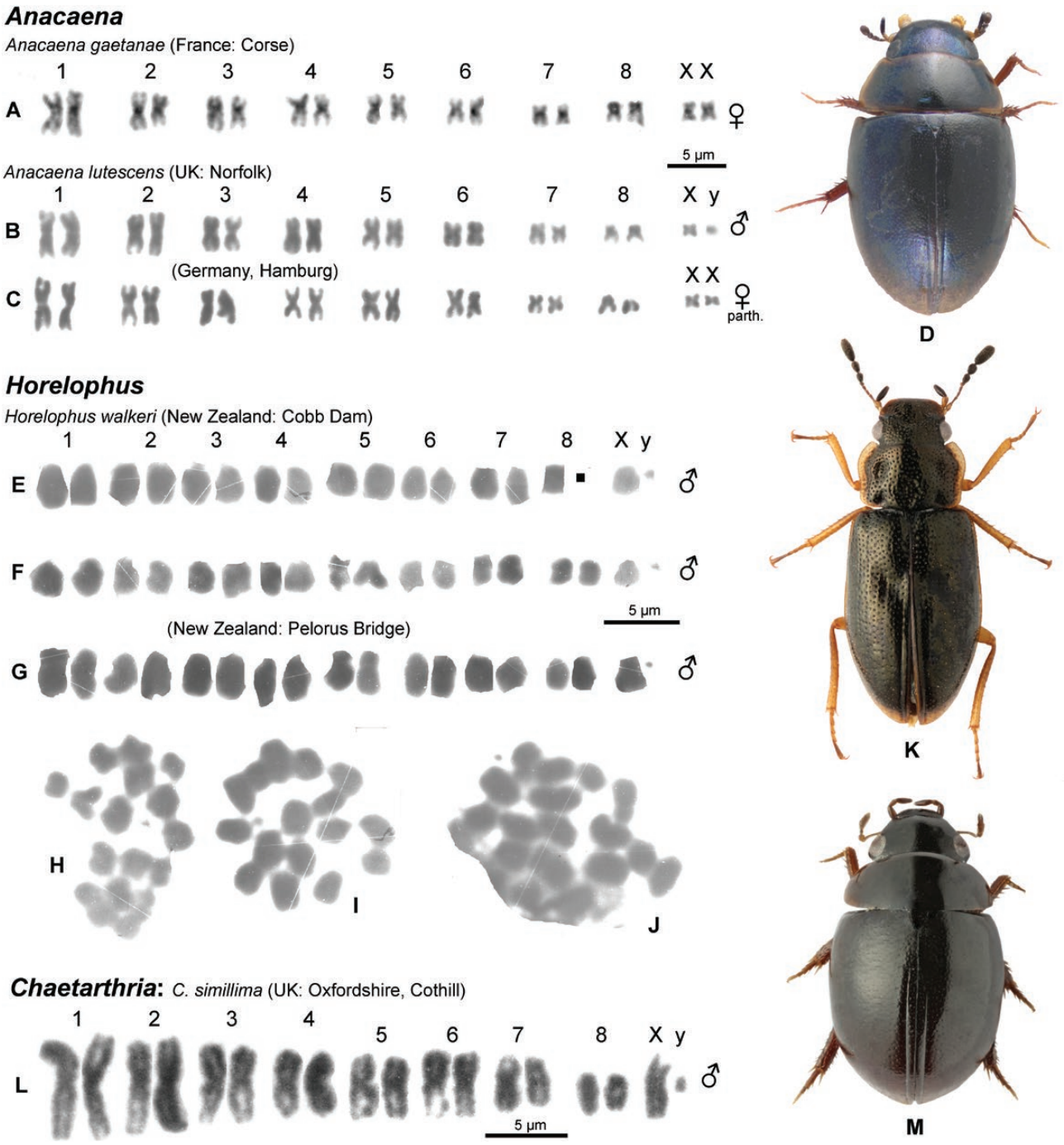

Figure 7. Mitotic karyotypes of Chaetarthriinae from the midgut. A–C: *Anacaena*: (A) *Anacaena gaetanae*; (B–C) *Anacaena lutescens* (B, sexually reproducing specimen; C, parthenogenetic female). E–J, *Horelophus walkeri*. L, *Chaetarthria simillima*. A, C-banded; B, C, E–J, L, without treatment. Habitus figures: (D) karyotyped voucher of *Anacaena gaetanae*; (K) *Horelophus walkeri*, from Fikáček *et al.* (2012a); (M) *Chaetarthria seminulum*, from Fikáček & Liu (2019).

length of pair 1. The metacentric X chromosome is the smallest in the nucleus, apart from the dot-like y.

Note: The monotypic genus *Helocombus* (LeConte, 1855) was synonymized with *Cymbiodyta* by Toussaint & Short (2019). The karyotype of its only species, *Cymbiodyta bifidus* (LeConte, 1855), was examined by Smith (1960) and reported to be $2n = 16 + Xy_p$ (♂), corresponding to our findings in the European *C. marginella*.

GENUS *ENOCHRUS* THOMSON, 1859
(FIGS 8B–J, 9–10)

Material examined: Enochrus (s.s.) *melanocephalus* (Olivier, 1792): 2 spec. (BMNH): United Kingdom, Surrey, Egham, R. Angus lgt.

Enochrus (Methydrus) affinis (Thunberg, 1794): 2 spec. (BMNH): United Kingdom, Hampshire, New Forest, R. Angus lgt.

Enochrus (Methydrus) coarctatus (Gredler, 1863): 1 spec. (BMNH): United Kingdom, Dorset, Studland Heath, R. Angus lgt.

Enochrus (Methydrus) nigritus (Sharp, 1872): 1 spec. (BMNH): Spain, Peñalara, R. Angus lgt.; 1 spec. (BMNH): United Kingdom, Norfolk, East Walton, R. Angus lgt.; 1 spec. (BMNH): Greece, Corfu, R. Angus lgt.

Enochrus (Methydrus) morenae (Heyden, 1870): 1 spec. (BMNH): Spain, Plasencia, R. Angus lgt.

Enochrus (Methydrus) sauteri d'Orchymont, 1913: 2 spec. (NMPC): Taiwan, 4.8 km SEE of Tonglin, Beikeng Creek Trail, 24.04791°N 120.78434°E, 3.x.2018, Fikáček, Hu, Liang & Liu lgt.

Enochrus (Lumetus) bicolor (Fabricius, 1792): 1 spec. (BMNH): United Kingdom, Sussex, Cuckmere, R. Angus lgt.

Enochrus (Lumetus) ochropterus (Marsham, 1802): 2 spec. (BMNH): United Kingdom, Dorset, Studland Heath, R. Angus lgt.; 2 spec. (BMNH): Germany, Niedersachsen, Staatsforest Gohrde, R. Angus lgt.

Enochrus (Lumetus) testaceus (Fabricius, 1801): 2 spec. (BMNH): United Kingdom, Norfolk, East Walton, R. Angus lgt.; 2 spec. (BMNH): Sweden, Öland, R. Angus lgt.

Enochrus (Lumetus) quadripunctatus (Herbst, 1797): 2 spec. (BMNH): United Kingdom, Norfolk, East Walton, R. Angus lgt.; 2 spec. (BMNH): Germany, Schleswig-Holstein, Ratzeburg, R. Angus lgt.

Enochrus (Lumetus) fuscipennis (Thomson, 1884): 2 spec. (BMNH): United Kingdom, Cumbria, Drigg, R. Angus lgt.; 2 spec. (BMNH): United Kingdom, Scotland, Ayrshire, Lochton loch, R. Angus lgt.; 3 spec. (BMNH): Germany, Schleswig-Holstein, Ratzeburg, R. Angus lgt.; 2 spec. (BMNH): Spain, Provincia de Santander, Embalse de Ebro, R. Angus lgt.; 2 spec. (BMNH): Denmark, Rømø Island, R. Angus lgt.; 2 males (BMNH): Denmark, Rømø Island, R. Angus lgt.*Enochrus (Lumetus) halophilus* (Bedel, 1878): 2 spec. (BMNH): United Kingdom, Sheppey, Kent, R. Angus lgt.; 2 spec. (BMNH): United Kingdom, Bembridge, Isle of Wight, R. Angus lgt. 2 spec. (BMNH): Spain, Albacete, Pétrola, P. Arribas lgt.

Karyotypes: $2n = 16 + Xy_p$ (♂). *Enochrus* s.s. (Fig. 8H) has autosomal pairs 3, 6 and 8 subacrocentric, pair 3 is heterozygous for an extra C-band. The other pairs, and the X chromosome, are metacentric, with well-developed centromeric C-bands. The subgenera *Methydrus* and *Lumetus* show no obvious features in their karyotypes, whereas the Taiwanese *Enochrus sauteri* (Fig. 8G) is unusual in having autosome pair 1 clearly twice the lengths of the others, which are all of the similar size.

The karyotypes appear remarkably uniform with some apparent species differences. In European *Methydrus*, *Enochrus coarctatus* (Fig. 8C) differs from *Enochrus affinis* (Fig. 8B) and *Enochrus nigritus* (Fig. 8D–E) in having autosome pairs 7 and 8, and the X chromosome, subacrocentric, whereas in the others they are metacentric. No differences were found between Spanish (dark brown) and English pale coloured specimens of *Enochrus nigritus* (compare Fig. 8D and E). In *Lumetus*, English *Enochrus halophilus* (Fig. 9F–K) differs from *Enochrus quadripunctatus* and *Enochrus fuscipennis* (Fig. 10) in having autosome pairs 7 and 8, and the X chromosome acrocentric, with the centromeric C-bands only about half the size of those of the metacentric autosomes. However, in Spanish *Enochrus halophilus*, which are inseparable from English material by mitochondrial DNA (P. Arribas, unpubl.), all the chromosomes (apart from the dot-like Y) are metacentric with C-bands of the same size as in *Enochrus halophilus* (Fig. 9H–K). The separation of *Enochrus fuscipennis* from *Enochrus quadripunctatus* is also problematic as the mitochondrial DNA reveals *Enochrus fuscipennis* intermixed with *Enochrus quadripunctatus* (P. Arribas, unpubl.). The situation is made even more complicated by material from Ratzeburg (Germany). This material included clear *Enochrus quadripunctatus* whose karyotype matched that of English specimens (as in Fig. 10A–C) and also apparent *Enochrus fuscipennis* whose karyotype differed in the submetacentric autosome 1 and the clearly smaller X chromosome (Fig. 10D–G). The material from the Rømø Island (Denmark) (Fig. 10H–I) comprised two males whose karyotypes could only be understood if they were hybrids (but not F1) between western and Ratzeburg-type populations of *Enochrus fuscipennis*. Both males have small X chromosomes and one has autosome 1 heterozygous for western and Ratzeburg forms, whereas the other has both replicates of the western form. One male has a B-chromosome and both have normal first metaphase of meiosis.

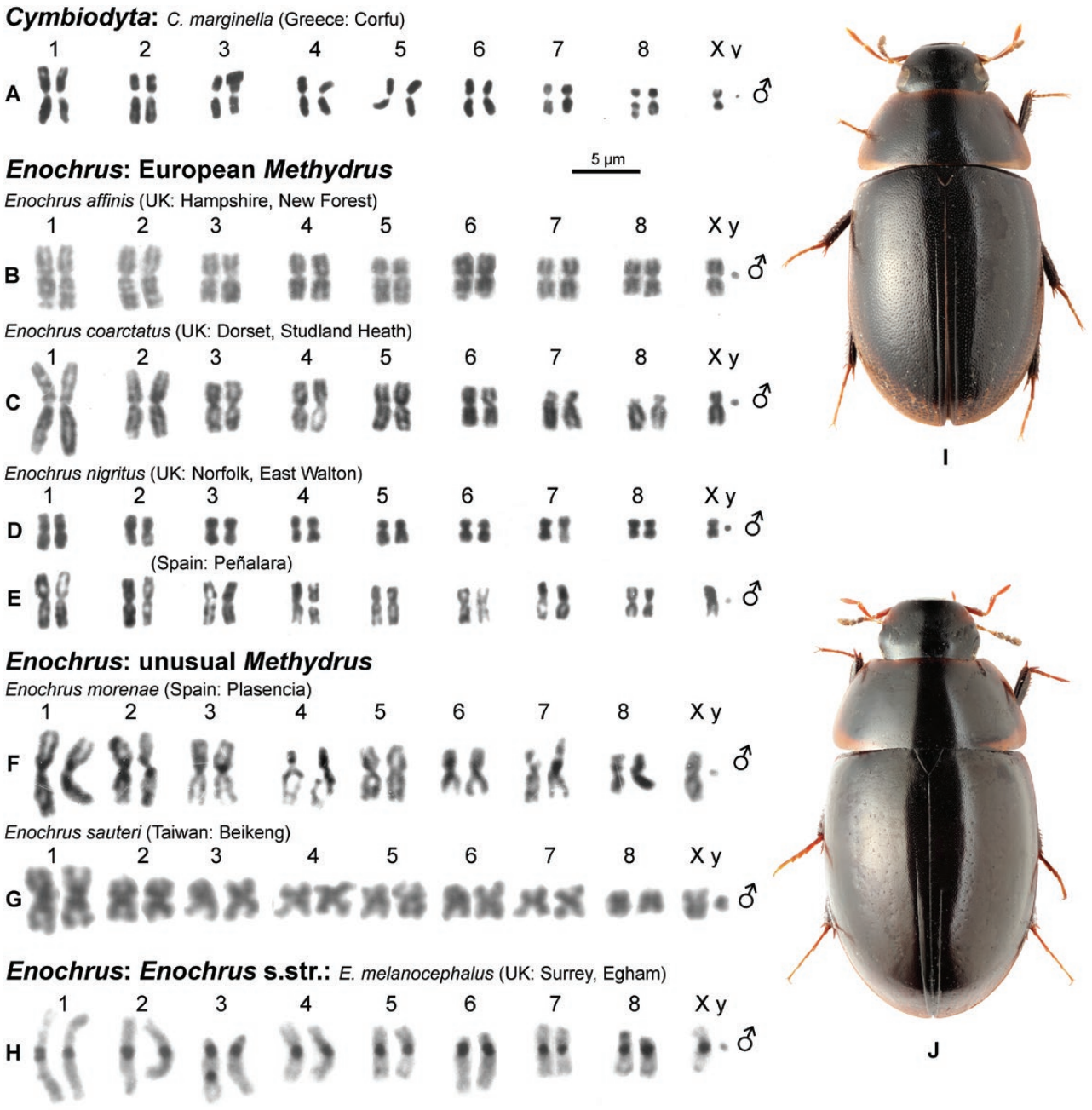

Figure 8. Mitotic karyotypes of the Enochrinae. A, *Cymbiodyta marginella*, embryo. B–D, European usual-looking species of *Enochrus* (*Methydrus*) from embryos: (B) *Enochrus affinis*; (C) *Enochrus coarctatus*; (D–E) *Enochrus nigritus*. F–G, unusual species assigned at the moment to *Enochrus* (*Methydrus*): (F) *Enochrus morenae*, midgut; (G) *Enochrus sauteri*, midgut. H, *Enochrus* (s.s.) *melanocephalus*, embryo. A–G, without treatment. H, C-banded. Habitus figures: (I) *Cymbiodyta marginella*; (J) *Enochrus morenae*.

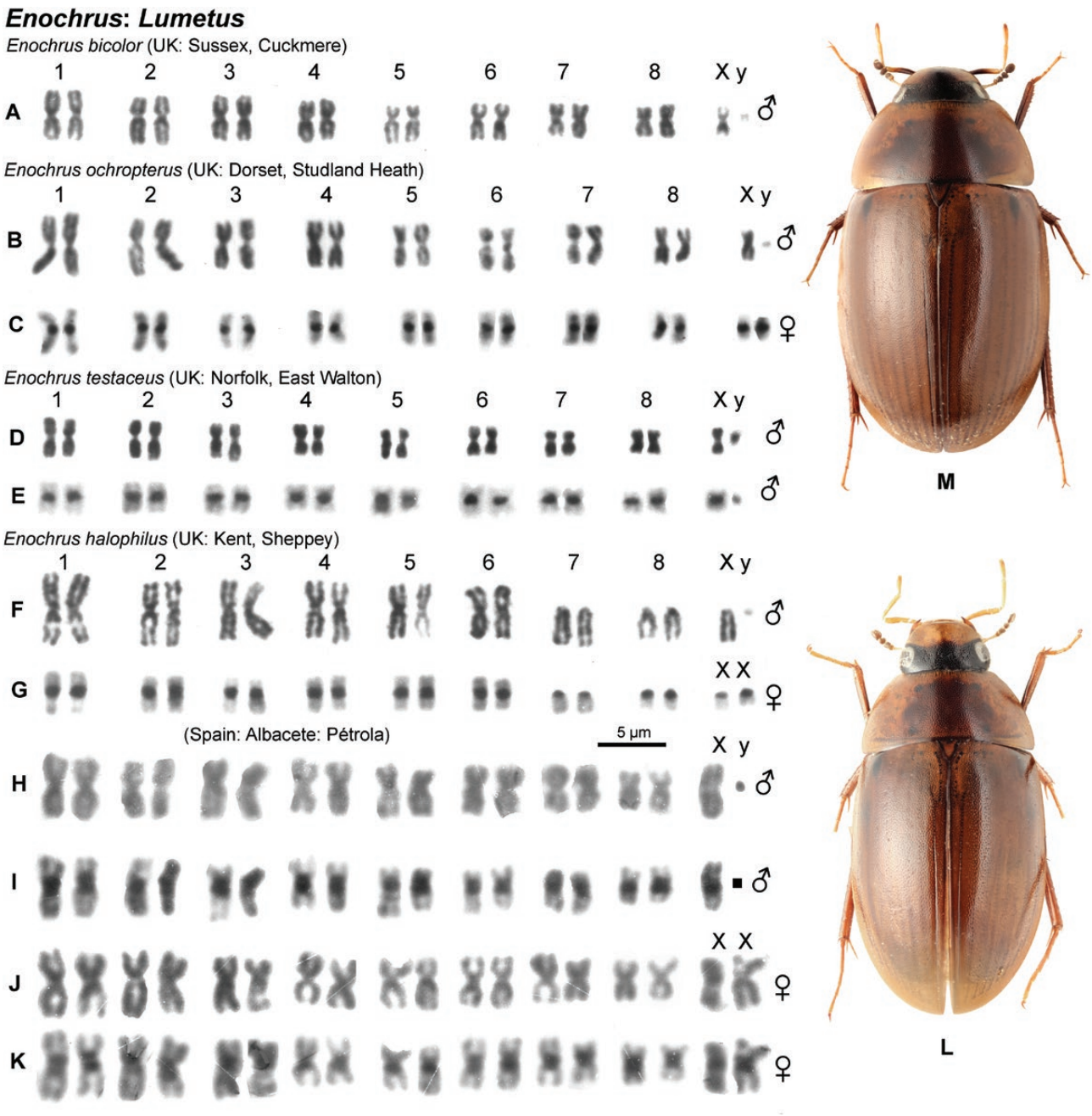

Figure 9. Karyotypes of *Enochrus* (*Lumetus*), mitosis from embryos. A, *Enochrus bicolor*. B–C, *Enochrus ochropterus*. D–E, *Enochrus testaceus*. F–K, *Enochrus halophilus*. A, B, D, F, H, I, without treatment. C, E, G, K, C-banded. Habitus figures: (L) *Enochrus* (*Lumetus*) *testaceus*; (M) *Enochrus* (*Lumetus*) *halophilus*.

SUBFAMILY ACIDOCERINAE
GENUS *AGRAPHYDRUS* RÉGIMBART, 1903
(FIG. 11A–C, M)

Material examined: Agraphydrus decipiens Minoshima, Komarek & Ohara, 2015: 1 male, 1 female (BMNH), 1 male (NMPC): Taiwan, 4.8 km SEE of Tonglin, Beikeng Creek Trail, 24.04791°N 120.78434°E, 3.x.2018, under leaves on a wet rock, lgt. M. Fikáček, H.-C. Liu, F.-S. Hu & W.-R. Liang.

Agraphydrus variabilis Komarek & Hebauer, 2018: 1 female (BMNH): Taiwan, 4.8 km SEE of Tonglin, Beikeng Creek Trail, 24.04791°N 120.78434°E,

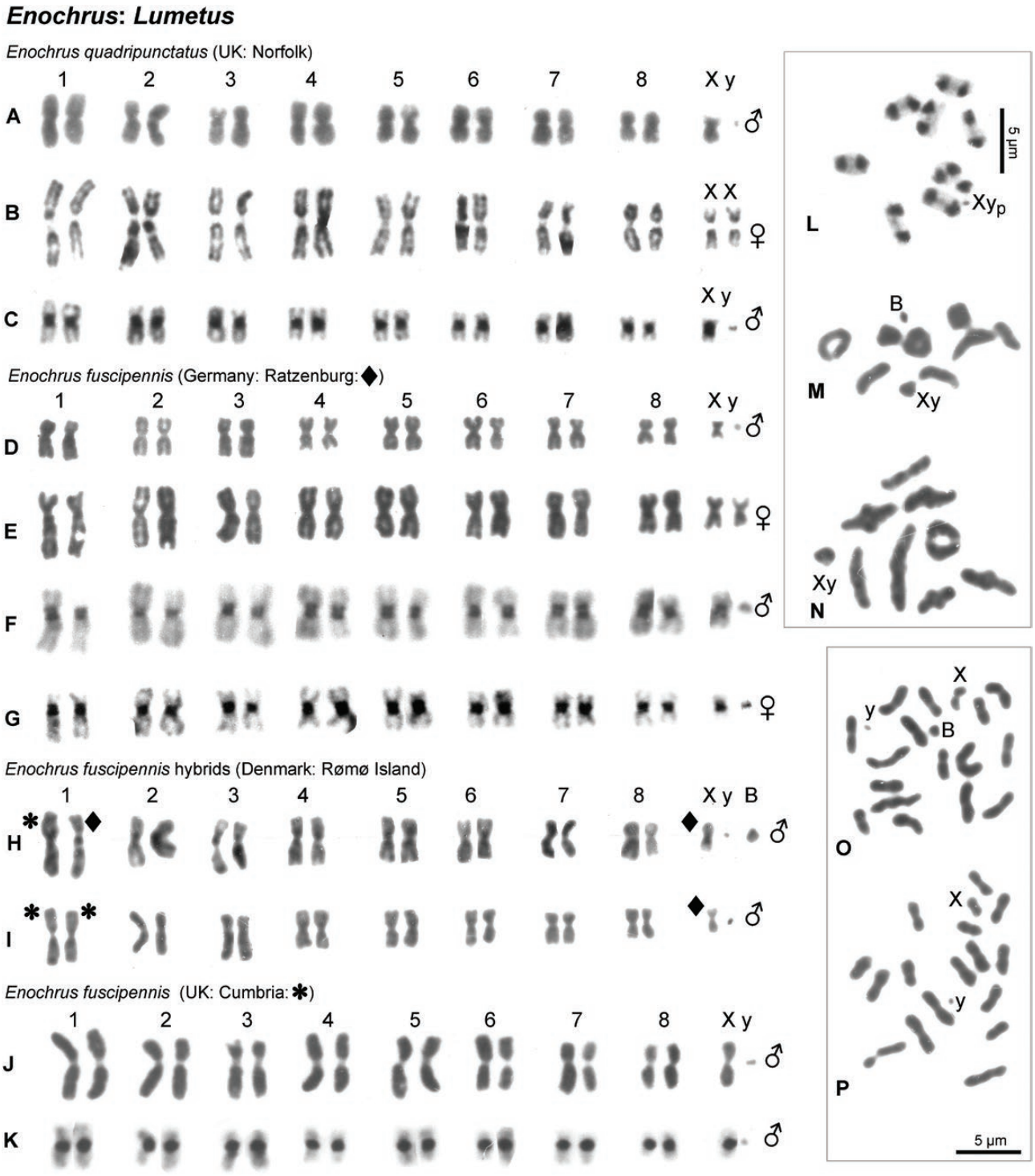

Figure 10. Karyotypes of *Enochrus* (*Lumetus*). A–C, *Enochrus quadripunctatus*, mitosis, midgut. D–K, *Enochrus fuscipennis*, mitosis, midgut (H–I, specimens from Denmark, Rømø Island with the karyotypes indicating their hybrid origin). L–N, meiotic metaphase I from testes (L, *Enochrus quadripunctatus*, UK: East Walton, Norfolk; M–N, *Enochrus fuscipennis*, Denmark: Rømø Island). O–P, *Enochrus fuscipennis*, testes, mitotic metaphase from the same specimens as in (H–I). A, B, D, E, H, I, J, M–P, without treatment. C, F, G, K, L, C-banded.

3.x.2018, in gravel at side of a river, lgt. M. Fikáček, H.-C. Liu, F.-S. Hu & W.-R. Liang.

Karyotype: $2n = 16 + Xy$ (♂). The autosomes show a gradual decrease in size along the karyotype so that pair 8 is about half the length of pair 1. In *Agraphydrus decipiens* (Fig. 11A–B), pairs 7 and 8, and the X chromosome, are acrocentric, whereas the rest (apart for the dot-like Y chromosome) are metacentric. The sex chromosomes of *Agraphydrus variabilis* (Fig. 11C) cannot be identified as only females are available. The smallest chromosome is acrocentric, the rest metacentric. C-bands are confined to the centromeres and are small, especially in *Agraphydrus decipiens*.

Genus *Helochares* Mulsant, 1844
(Fig. 11D–L, N)

Material examined: Helochares lividus (Forster, 1771): 2 spec. (BMNH): United Kingdom, Somerset, Shapwick, R. Angus lgt.; 1 spec. (BMNH): Spain, Provincia de Santander, Embalse de Ebro, R. Angus lgt.; 1 spec. (BMNH): Italy, Sardinia, Nuoro Province, R. Angus lgt.

Helochares obscurus (Müller, 1776): 1 spec. (BMNH): Sweden, Öland, R. Angus lgt.

Helochares punctatus Sharp, 1869: 3 spec. (BMNH): United Kingdom, Surrey, Chobham, R. Angus lgt.; 2 spec. (BMNH): United Kingdom, Hampshire, New Forest, R. Angus lgt.

Helochares sauteri d'Orchymont, 1943: 3 spec. (NMPC): Taiwan, 2.5 km E of Tonglin, 24.06288°N 120.76323°E, 3.x.2018, M. Fikáček, F.-S. Hu, W.-R. Liang & H.-C. Liu lgt.

Karyotype: $2n = 16 + Xy_p$ (♂). In *Helochares lividus* (Fig. 11D–E), pairs 1–6 are metacentric, whereas pairs 7, 8 and the X are submetacentric; there are small centromeric C-bands on all the chromosomes except the Y. In *Helochares obscurus* (Fig. 11F), pairs 1–7 are metacentric, pair 8 is subacrocentric and the X chromosome submetacentric. In *Helochares punctatus* (Fig. 11G–H), the autosomes are all metacentric, the X chromosome is submetacentric and all chromosomes possess large centromeric C-bands. Some *H. punctatus* appear to have up to three dot-like Y chromosomes; however, the additional two dots are probably B-chromosomes. *Helochares sauteri* from Taiwan (Fig. 11L) differs from the other species in its small acrocentric X chromosome, only half the length of the smallest autosome.

Subfamily Cylominae
Genus *Adolopus* Sharp, 1884
(Fig. 12A–C)

Material examined: Adolopus sp. 1: 4 males, 3 females (NMPC): New Zealand: Stewart Island, Northwest Circuit Tk. at Kaipipi Bay, rotten longs/twigs in sparse hardwood forest with tree ferns, 46°53.88'S 168°4.31'E, 20 m a.s.l., 21.i.2016, M. Seidel & M. Fikáček lgt. (2016-NZ017).

Adolopus sp. 2: 1 male (NMPC): New Zealand, Waikato (WO), Pirongia Forest Park, Ruapane Link Track (lower part), 37.966°S 175.144°E, 235 m,18–21.xi.2016, M. Fikáček & M. Seidel lgt. (MM02).

Karyotype: $2n = 22 + Xy$ (♂). In the Kaipipi Bay species (Fig. 12B), autosome pair 1 is the longest, and remaining autosomes gradually decrease in length so that pair 11 is about a quarter of the length of pair 1. All autosomes are metacentric to submetacentric. The X chromosome is metacentric, about as long as autosome pair 5, and the Y chromosome is dot-like. *Adolopus* from Pirongia (Fig. 12A) differs from that of Kaipipi Bay by subacrocentric pairs 7–9 and the acrocentric X chromosome slightly longer than autosome pair 2.

Note: The identification of the examined specimens is impossible at the moment; however, the DNA data (Seidel, unpubl.) indicate that they represent two different species.

Genus *Cyloma* Sharp, 1872
(Fig. 12D–H)

Material examined: Cyloma guttulatus Sharp, 1884: 1 female (NMPC): New Zealand: Fiordland (FD), Borland Road 24 km NWW of Monowai, baited pitfall traps (rotten squid) in degraded remnants of *Nothofagus* forest with numerous rotten logs, 45°41.13'S 167°20.29'E, 320 m, 27.i.2016, M. Seidel, V. Sýkora & M. Fikáček lgt. (2016-NZ024).

Cyloma sp.: (undescribed species illustrated in Fig. 12H). 1 male, 1 female (NMPC): New Zealand: Fiordland (FD), Borland Road 24 km NWW of Monowai, on dead possum, 45°41.13'S 167°20.29'E,

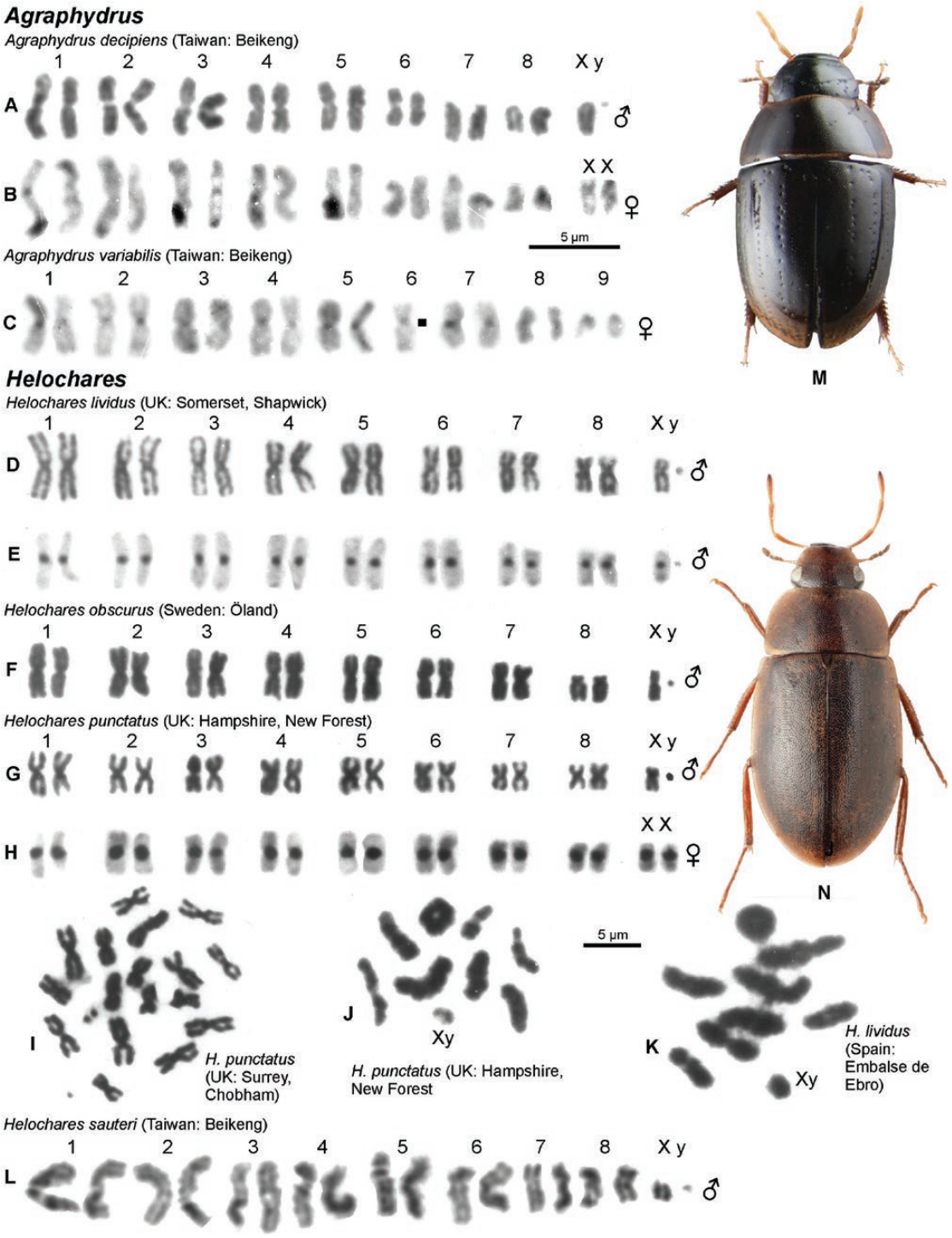

Figure 11. Karyotypes of the Acidocerinae. A–C, *Agraphydrus*, mitotic metaphase, midgut: (A–B) *Agraphydrus decipiens*; (C) *Agraphydrus variabilis*. D–L, *Helochares*: (D–E) *Helochares lividus*, mitosis, midgut; (F) *Helochares obscurus*, mitosis, midgut; (G–H) *Helochares punctatus*, mitosis, midgut; (I) *Helochares punctatus*, karyotype of male embryo with multiple y-chromosomes; (J–K) meiotic metaphase I from testes (J, *Helochares punctatus*; K, *Helochares lividus*); (L) *Helochares sauteri*, mitosis, midgut. A, D, F, G, I–L, without treatment. B, C, E, H, C-banded. Habitus figures: (M) *Agraphydrus decipiens*, from Minoshima, Komarek, & Ôhara 2015; (N) *Helochares obscurus*.

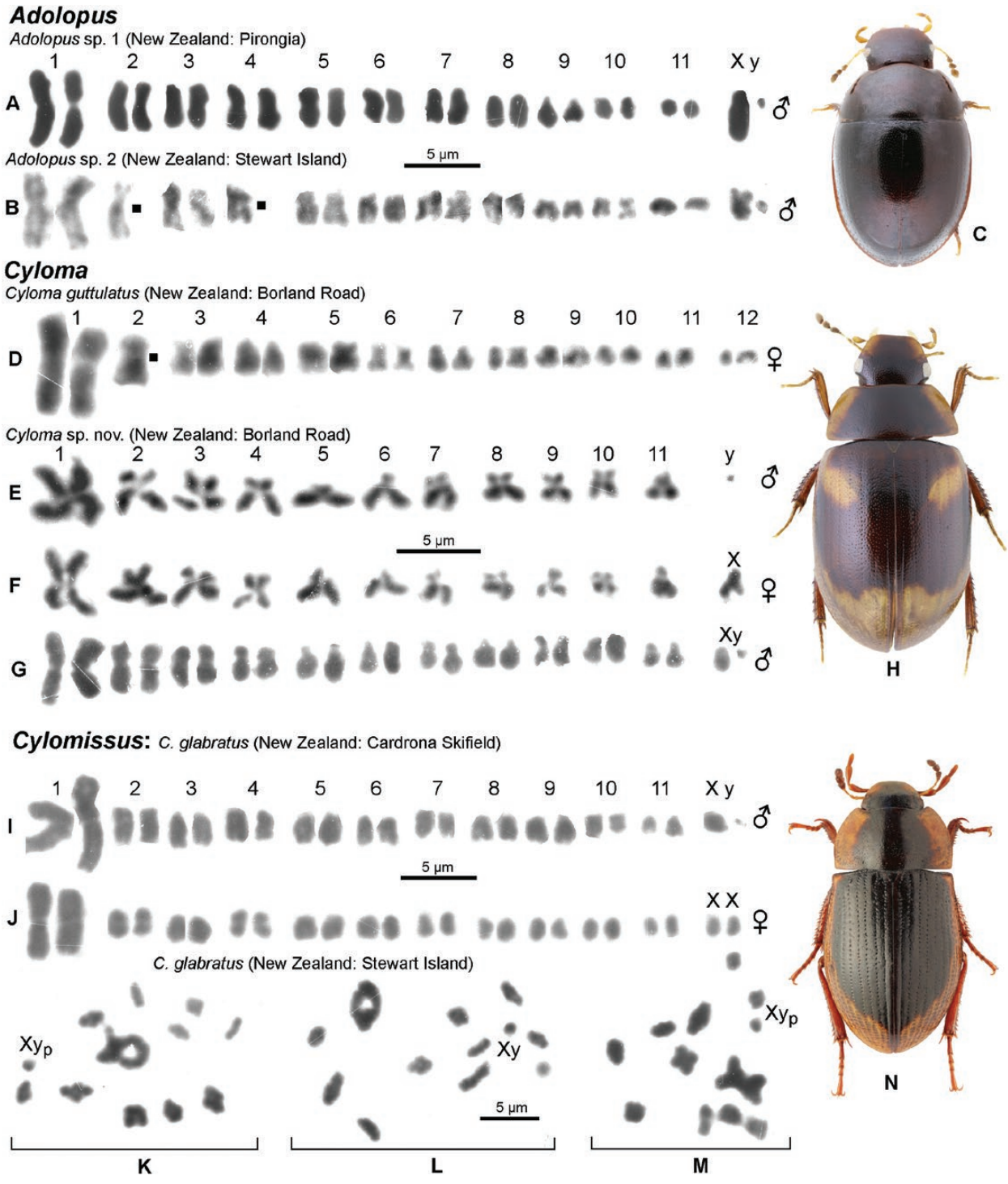

Figure 12. Karyotypes of the Cyclominae, without treatment. A–B, *Adolopus* sp., mitosis from midgut. D, *Cyloma guttulatus*, mitosis from midgut. E–G, *Cyloma* sp. (E–F, meiotic first metaphase; G, testes, mitosis;). I–M, *Cylomissus glabratus*: (I–J) mitotic metaphase from midgut; (K–M) meiotic metaphase I from testes. Habitus figures: (C) *Adolopus* sp.; (H) *Cyloma* sp., specimen collected with karyotyped voucher; (N) *Cylomissus glabratus*, from Minoshima *et al.* (2015).

320 m, 27.i.2016, M. Seidel, V. Sýkora & M. Fikáček lgt. (2016-NZ024).

Karyotype: $2n = 22 + Xy$ (♂). In *Cyloma guttulatus*, chromosome pairs 1–3 and 5 are metacentric, with pair 2 half the size of pair 1, and the others decreasing in size from half the length of pair 2 to about quarter of the size of pair 2 (Fig. 12D). The X chromosomes cannot be identified. *Cyloma* sp. (Fig. 12E–G) has a similar karyotype to *Cyloma guttulatus*; however, autosome pair 2 is about two-thirds of the length of pair 1 and the smallest autosomes, and the X chromosome are about a quarter of the length of pair 1.

Genus *Cylomissus* Broun, 1903
(Fig. 12I–N)

Material examined: Cylomissus glabratus Broun, 1903: 1 male, 1 female (NMPC): New Zealand: Otago Lakes, Little Meg headwaters at Cardrona Skifield Rd., stream collecting in small exposed streamlets (stony and mossy), 44°52.60'S 168°57.65'E, 1280 m, 5.xii.2017, M. Fikáček, D. Sadílek & V. Sýkora lgt. (2017-NZ63). 1 male (NMPC): New Zealand: Stewart Island, Fern Gully W of Oban, at bridge over Mill Creek, 17–21.i.2016, 46°53.52'S 168°6.00'E, 45 m, M. Seidel, V. Sýkora & M. Fikáček lgt. (2016-NZ004).

Karyotype: $2n = 22 + Xy$ (♂). Autosome pair 1 is 3–4 times the length of pair 2, whereas the remaining pairs have a gradual decrease in length along the karyotype, with pair 11 about half the length of pair 2, the X chromosome is only slightly longer than pair 11. The Y chromosome is dot-like. Examined specimens from both localities have a similar karyotype.

Genus *Exydrus* Broun, 1886
(Fig. 13A, D, I)

Material examined: Exydrus gibbosus Broun, 1886: 1 female (NMPC): New Zealand: Wellington (WL), Tararua Range, 1 km W of Tituraea Dam, start of Greens Rd., 40.4295°S 175.66064°E, 145 m, 26.xi.2016, fragment of broadleaf forest with sparse understory with ferns and *Pandanus* Parkinson: sifting, M. Fikáček & M. Seidel lgt. (MM31).

Karyotype: $2n = 30$ (♀). The only nucleus obtained has 29 chromosomes and the karyogram shows a serious size mismatch in pair 3 so there should be at least 30 chromosomes in the karyotype, presumably 14 pairs of autosomes plus the sex chromosomes. The smallest chromosome appears heavy and almost single-stranded; however, this is almost certainly because the two chromatids of a metacentric are lying on top of each other. This is frequent with small chromosomes. There is gradual decrease in length along the karyotype, with no pair strikingly longer than the others. The smallest pair is about a third of the length of the longest pair. Most of the chromosomes are metacentric or submetacentric; however, pairs 6–9 are subacrocentric.

Genus *Rygmodus* White, 1846
(Fig. 13F–H, J)

Material examined: Rygmodus modestus White, 1846: 1 male (NMPC): New Zealand: Rangitikei (RI), Ruahine Forest Park, Kashmir Road, sweeping of flowering *Gaultheria* L. and *Brachyglottis repanda* J.R.Forster & G.Forster, 39.94°S 176.16859°E, 655 m, 25.xi.2016, M. Fikáček & M. Seidel lgt. (MM25). 1 female (NMPC): New Zealand: Taranaki, 0.2 km S of Pukeiti Garden, 9 km E of Okato, lowland *Nothofagus* forest, flight interception trap, 370 m, 173.98°S 39.20°E, 10.xii.2016, lgt. M. Fikáček & M. Seidel (MM09).

Karyotype: $2n = 13 + Xy_p$ (♂). There is a gradual decrease in length along the karyotype, with pair 13 about a quarter of the length of pair 1. Most of the chromosomes, including the X, are acrocentric or subacrocentric. The Y chromosome is small, though not dot like, and first metaphase of meiosis shows the typical parachute association, Xy_p.

Genus *Tormissus* Broun, 1893
(Fig. 13B–C, E)

Material examined: Tormissus magnulus Broun, 1893: 1 male (NMPC): New Zealand, Wellington (WL), Tararua Range, 1 km W of Tituraea Dam, start of Greens Rd., fragment of broadleaf forest with sparse understory with ferns and *Pandanus*: pitfall trap, 40.4295°S 175.66064°E, 145 m, 28.xi.2016, M. Fikáček & M. Seidel lgt. (MM31). 1 male (NMPC): New Zealand: Wellington, Wright Hill Reserve, along unnamed stream along Deliverance Tk., 41.29683°S 174.73285°E, 190 m, 18.xi.2017, D. Sadílek lgt. (2017-NZ07; DNA extraction NZ718).

Karyotype: $2n = 26 + Xy_p$ (♂). Based on the specimen from Tituraea Dam (Fig. 13B, C, E), the karyotype comprises 13 pairs of autosomes plus Xy sex chromosomes. The specimen from Wellington provided two suitable mitotic nuclei and one meiotic nucleus,

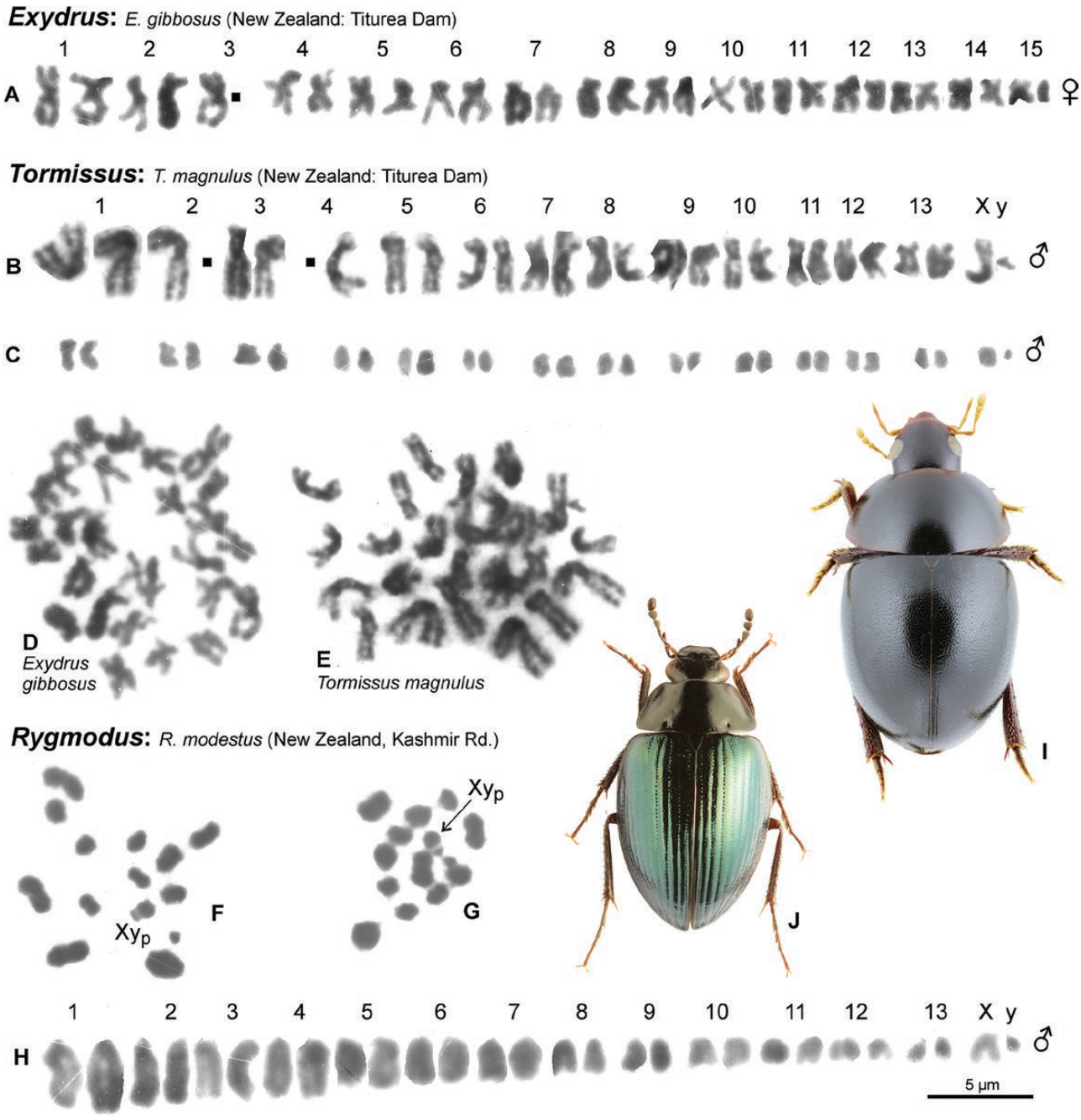

Figure 13. Karyotypes of the Cylominae, without treatment. A, D, *Exydrus gibbosus*, mitosis, midgut. B–C, E, *Tormissus magnulus*, mitosis, midgut. F–H, *Rygmodus modestus* (F–G, meiotic metaphase I, testes; H, mitotic karyotype, midgut). Habitus figures: (I) *Exydrus gibbosus*; (J) *Rygmodus modestus*, from Minoshima *et al.* (2018).

all showing one chromosome pair less (not illustrated here), despite the Titurea and Wellington specimens being conspecific as confirmed by their DNA barcodes. The Wellington specimen clearly shows the Xy_p configuration of sex chromosomes. We suppose that the lower number of chromosomes in the Wellington specimen is caused by a loss of a chromosome pair during preparation.

Subfamily Sphaeridiinae
Tribe Omicrini
Genus *Noteropagus* d'Orchymont, 1919
(Fig. 14A–B, G)

Material examined: Noteropagus sp.: (unidentified species illustrated in Fig. 14G). 1 male (NMPC): Taiwan: Nantou County, Yushan National Park, Dongbu Scenic Area, Dong Bu 5.4 km SE of Heshe, rotten banana trunks on the margin of a village, 23.5610°N 120.93044°E, 15.v.2018, M. Fikáček, W.-R. Liang, H.-C. Liu & Y. Minoshima lgt. (2018-TW33).

Karyotype: $2n = 22$. Two nuclei from testis show probably the meiotic metaphase I with 11 bivalents. The sex bivalent cannot be recognized, although the smallest element in Figure 14A appears asymmetrical. The remaining bivalents suggest that the autosomes are all more or less similar in size.

Genus *Omicrogiton* d'Orchymont, 1919
(Fig. 14C–D, H)

Material examined: Omicrogiton insularis d'Orchymont, 1919: 1 male, 1 female (BMNH): Taiwan, 4.8 km SEE of Tonglin, Beikeng Creek Trail, 24.04791°N 120.78434°E, 3.x.2018, rotten banana stem, M. Fikáček, H.-C. Liu, F.-S. Hu & W.-R. Liang lgt.

Karyotype: $2n = 24 + Xy$ (♂). Autosome pairs 1–3 and the X chromosome are metacentric and clearly larger than the other pairs. Pairs 4 and 7–12 are subacrocentric to acrocentric, and the smallest autosome is about a quarter the length of the largest. The Y chromosome is dot like.

Genus *Paromicrus* Scott, 1913
(Fig. 14E–F, I)

Material examined: Paromicrus sp.: [unidentified species likely close to *Paromicrus affinis* (Fig. 14I)]: 2 females (BMNH): Taiwan, 4.8 km SEE of Tonglin, Beikeng Creek Trail, 24.04791°N 120.78434°E, 3.x.2018, rotten banana stem, M. Fikáček, H.-C. Liu, F.-S. Hu & W.-R. Liang lgt.

Karyotype: $2n = 26$ (♀). All the chromosomes are subacrocentric to acrocentric and there is a gradual decrease in size along the karyotype, with the smallest chromosomes about half of the length of the largest. The X chromosomes cannot be identified.

Tribe Coelostomatini
Genus *Coelostoma* Brullé, 1835
(Fig. 15A–C, H)

Material examined: Coelostoma orbiculare (Fabricius, 1775): 1 male, 2 females (BMNH): United Kingdom, Norfolk, East Walton, R. Angus & F. Shaarawi lgt.

Karyotype: $2n = 26 + Xy$ (♂). The autosomes and the X chromosome are metacentric to submetacentric with strong centromeric C-bands. Autosome pair 6 has a distinct subterminal secondary constriction on its long arm. The Y chromosome is dot like, and there may be two small B-chromosomes in the examined specimens. All the nuclei are from eggs taken from a cocoon spun by one of the females. In total, 16 male and 6 female nuclei were photographed. The two small B-chromosomes were present in only one male nucleus.

Genus Dactylosternum Wollaston, 1854
(Fig. 15D–G, I)

Material examined: Dactylosternum corbetti (Balfour-Browne, 1942): 1 male (NMPC): Taiwan, Nantou County, 4.8 km SSE of Tonglin, Beikeng Creek Trail, 24.04791°N 120.78434°E, 3.x.2018, M. Fikáček, F.-S. Hu, W.-R. Liang & H.-C. Liu lgt.

Dactylosternum flavicorne (Mulsant, 1844): 1 male, 1 female (BMNH): Dominican Republic, lowland rainforest, locality unknown, R. Angus lgt.

Karyotype: $2n = 26 + Xy$ (♂). In *Dactylosternum flavicorne* (Fig. 15D–F), the autosomes are metacentric to submetacentric, with the smallest pair about half of the length of the largest pair. The X chromosome is metacentric and the largest in the karyotype. All the chromosomes, except the dot-like Y, have large centromeric C-bands. The karyotype of *Dactylosternum corbetti* (Fig. 15G) has the autosomes and X chromosome metacentric to submetacentric and with smaller C-bands than in *Dactylosternum flavicorne*. Pair 1 is distinctly longer than the others, and the X chromosome is about as long as pair 5.

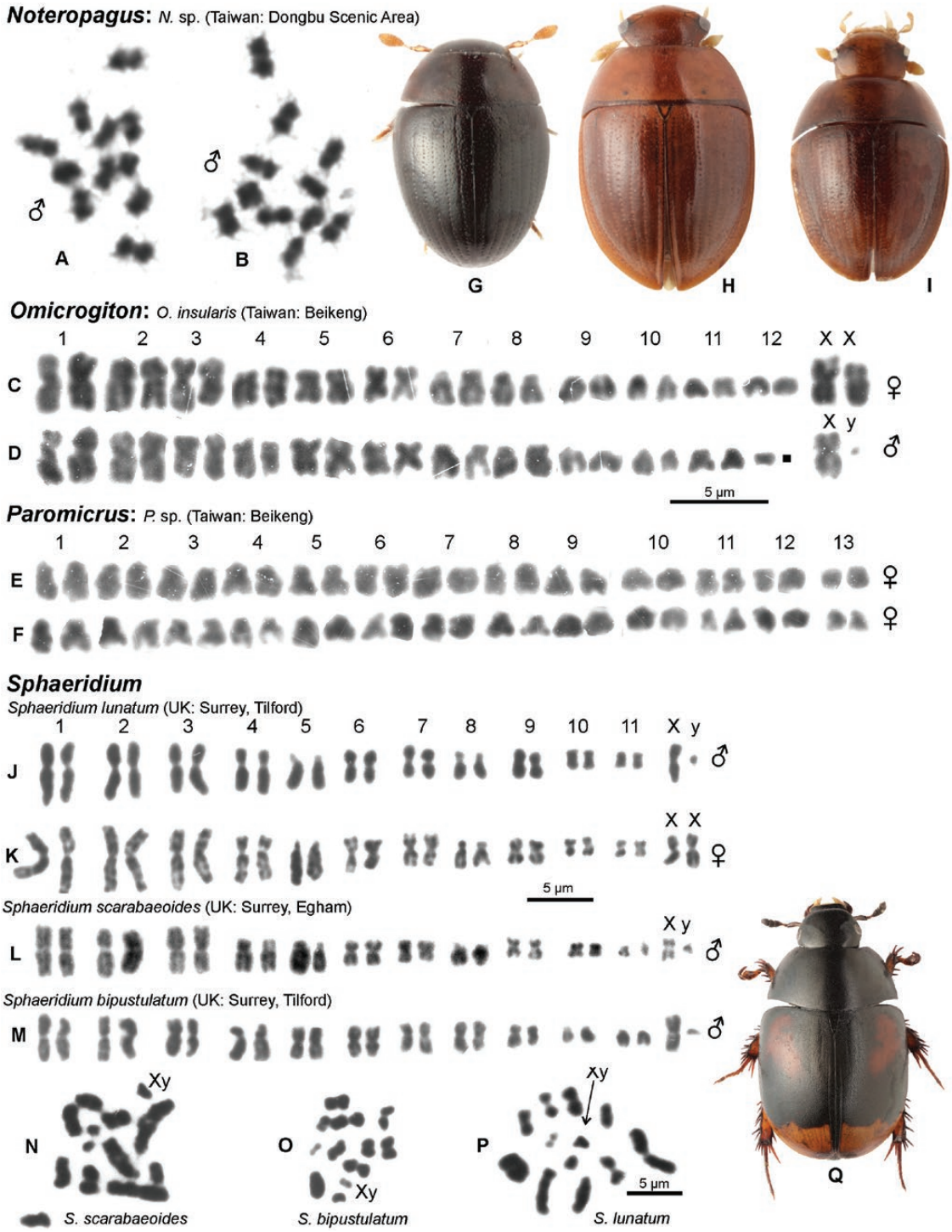

Figure 14. Karyotypes of the Omicrini and Sphaeridiini, without treatment. A–F, Omicrini: (A–B) *Noteropagus* sp. from Taiwan, meiotic karyotypes from testes; (C–D) *Omicrogiton insularis*, mitotic karyotype, midgut; (E–F) *Paromicrus* sp. from Taiwan, mitotic karyotype, midgut. G–I, habitus of examined specimens: (G) *Noteropagus* sp.; (H) *Omicrogiton insularis*; (I) *Paromicrus* sp. J–P, Sphaeridiini: *Sphaeridium;* (J–K) *Sphaeridium lunatum* (J, testes, mitosis; K, midgut, mitosis); (L) *Sphaeridium scarabaeoides*, mitosis, midgut; (M) *Sphaeridium bipustulatum*, mitosis, midgut; (N–P) meiosis, metaphase I, testes: (N) *Sphaeridium scarabaeoides*; (O) *Sphaeridium bipustulatum*; (P) *Sphaeridium lunatum*. Q, habitus of *Sphaeridium scarabaeoides.*

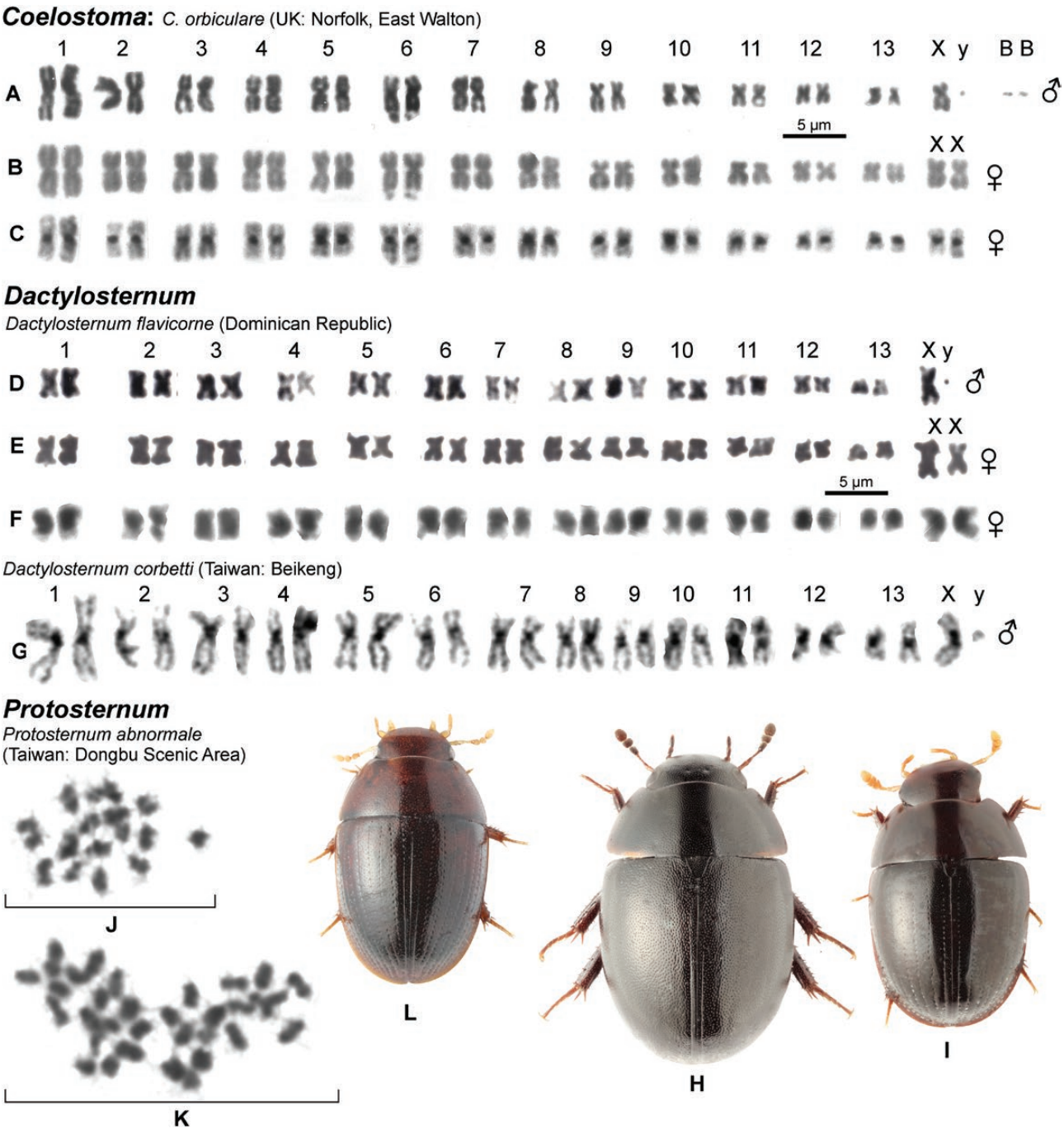

Figure 15. Karyotypes of the Coelostomatini and Protosternini. A–C, *Coelostoma orbiculare*, embryo (A, with B-chromosomes; B–C, without B-chromosomes). D–F, *Dactylosternum flavicorne*, embryo. G, *Dactylosternum corbetti*, mitosis, midgut. J–K, *Protosternum abnormale*, meiotic nuclei from testes. A–F, J, K, without treatment. G, C-banded. Habitus figures: (H) *Coelostoma orbiculare*; (I) *Dactylosternum corbetti*; (L) *Protosternum abnormale*, from Fikáček *et al.* (2018).

Tribe Protosternini
Genus *Protosternum* Sharp, 1890
(Fig. 15J–L)

Material examined: Protosternum abnormale (d'Orchymont, 1913): 1 male (NMPC): Taiwan: Nantou County, Yushan National Park, Dongbu Scenic Area, Dong Bu, 5.4 km SE of Heshe, rotten banana trunks on the margin of a village, 23.5610°N 120.93044°E, 15.v.2018, M. Fikáček, W.-R. Liang, H.-C. Liu & Y. Minoshima lgt. (2018-TW33).

Karyotype: $2n = 32$. The material to hand is from the testis and some nuclei at least, particularly that in Figure 15J, appear to be first of meiotic metaphase I and exhibit 16 bivalents. The karyotype hence likely comprise 15 pairs of autosomes plus Xy sex chromosomes.

Tribe Sphaeridiini
Genus *Sphaeridium* Fabricius, 1775
(Fig. 14J–Q)

Material examined: Sphaeridium scarabaeoides (Linnaeus, 1758): 2 spec. (BMNH): United Kingdom, Surrey, Egham, R. Angus & F. Shaarawi lgt.

Sphaeridium lunatum Fabricius, 1792: 2 spec. (BMNH): United Kingdom, Surrey, Tilford, R. Angus & F. Shaarawi lgt.

Sphaeridium bipustulatum Fabricius, 1781: 1 spec. (BMNH): United Kingdom, Surrey, Tilford, R. Angus & F. Shaarawi lgt.

Comments: Angus *et al.* (2000) studied the mitotic and meiotic karyotypes of four European species, *Sphaeridium bipustulatum*, *Sphaeridium lunatum*, *Sphaeridium marginatum* and *Sphaeridium scarabaeoides*. The published figures of karyotypes were of low quality, and we hence provide those of three species again here and add the meiotic figures as well.

Karyotype: $2n = 22 + Xy$ (♂). In the larger species, *Sphaeridium lunatum* (Fig. 14J–K) and *Sphaeridium scarabaeoides* (Fig. 14L), autosome pairs 4 and 5 are subacrocentric to acrocentric and pair 8 subacrocentric, with pair 11 also subacrocentric in *Sphaeridium scarabaeoides*. In the smaller species, *Sphaeridium bipustulatum* (Fig. 14M) and *Sphaeridium marginatum*, autosome pair 5 is metacentric and pairs 4 and 10 are subacrocentric. The X chromosome is medium sized metacentric and the Y is dot like in all species.

Tribe Megasternini
Genus *Cercyon* Leach, 1817
(Figs 16-17)

Material examined: Cercyon (Dicyrtocercyon) ustulatus (Preyssler, 1790): 1 male (BMNH): United Kingdom, Surrey, Egham, R. Angus lgt.

Cercyon (Paracercyon) analis (Paykull, 1798): 1 female (NMPC): Czech Republic, Bohemia. Černíky, 3.vi.2019, collecting in compost, V. Sýkora lgt.

Cercyon convexiusculus Stephens, 1829: 1 male, 1 female (BMNH): United Kingdom, Norfolk, East Walton, R. Angus lgt. 1 spec. (NMPC): Czech Republic, Zliv, 49.0794131°N 14.3895733°E, 24.iv.2019, V. Kolář lgt.

Cercyon sternalis (Sharp, 1890): 2 females (BMNH): United Kingdom, Norfolk, East Walton, R. Angus lgt.

Cercyon tristis (Illiger, 1801): 1 female (BMNH): United Kingdom, Norfolk, East Walton, R. Angus lgt.

Cercyon marinus Thomson, 1853: 1 male (BMNH): United Kingdom, Norfolk, East Walton, R. Angus lgt.

Cercyon impressus (Sturm, 1807): 2 males (BMNH): United Kingdom, Surrey, Egham, R. Angus lgt.

Cercyon obsoletus (Gyllenhal, 1808): 1 male (BMNH): United Kingdom, Surrey, Bookham Common, R. Angus & H. Gray lgt.

Cercyon lateralis (Marsham, 1802): 1 male (BMNH): United Kingdom, Windsor, Boveney, R. Angus & H. Gray lgt.; 1 male (BMNH): United Kingdom, Windsor, Windsor Deer Park, R. Angus & H. Gray lgt.

Cercyon haemorrhoidalis (Fabricius, 1775): 1 male (BMNH): United Kingdom, Windsor, Boveney, R. Angus & H. Gray lgt.; 2 males (BMNH): United Kingdom, Windsor, Windsor Deer Park, R. Angus & H. Gray lgt.

Cercyon melanocephalus (Linnaeus, 1758): 1 male, 1 female (BMNH): United Kingdom, Windsor, Windsor Deer Park, R. Angus & H. Gray lgt.

Karyotypes: The examination of the eleven species shown here indicates that the subgenus *Dicyrtocercyon* (with the only species *Cercyon ustulatus*; Fig. 16A) and *C. convexiusculus* of the *Cercyon convexiusculus* group (Fig. 16F–I) have the karyotype of $2n = 28 + Xy$ (♂). All nine remaining species (*Cercyon sternalis* and *Cercyon tristis* of the *Cercyon convexiusculus* group, *Cercyon analis* of the subgenus *Paracercyon* and other examined 'usual' European *Cercyon*) have the karyotype $2n = 22 + Xy$ (♂).

Cercyon ustulatus (Fig. 16A) has a large metacentric X chromosome, about the same size as autosome 6. The autosomes are a mixture of metacentric and subacrocentric ones, with pair 14 about a quarter of the length of pair 1.

Cercyon convexiusculus (Fig. 16F–I) has a small metacentric X chromosome about the same size as autosome pair 9. Autosome pairs 4, 10 and 12–14 are acrocentric to subacrocentric whereas the remaining pairs are metacentric to submetacentric. Interestingly, pair 1 is polymorphic for a pericentric inversion—the East Walton specimens are either homozygous acrocentric (Fig. 16F) or heterozygous (Fig. 16G, H) whereas the Czech specimen (Fig. 16I) is homozygous submetacentric.

The remaining species differ from each other in the relative length of the X chromosome: it is a long metacentric in *Cercyon lateralis* (Fig. 17B–E), *Cercyon haemorrhoidalis* (Fig. 17I–K) and *Cercyon melanocephalus* (Fig. 17L–N), and shorter but still metacentric in *Cercyon marinus* (Fig. 17A), *Cercyon obsoletus* (Fig. 17F, G) and *Cercyon impressus* (Fig. 17H). The X chromosome cannot be identified in *Cercyon analis* (Fig. 16B), *Cercyon tristis* (Fig. 16C) and *Cercyon sternalis* (Fig. 16D, E) because only females are available.

Note: The yet unpublished DNA-based molecular phylogeny of the Megasternini (Arriaga-Varela & Fikáček, unpubl.) revealed that the genus is polyphyletic. The groups used in Figures 16 and 17 correspond to these molecular findings, each of them represents a monophyletic DNA clade.

DISCUSSION

Cytogenetics of the Hydrophilidae

The karyotypes of the water scavenger beetles (Hydrophilidae) are striking for their numerical stability in the vast majority of the aquatic species, even though these span four subfamilies and six tribes. Nearly all the included species have 18 chromosomes ($2n = 16 + Xy_p$) (Table 1). The tribe Hydrophilini is an exception: the genera *Hydrophilus* and *Hydrochara* have 30 chromosomes ($2n = 28 + Xy_p$) whereas *Sternolophus* and *Tropisternus* retain 18 chromosomes. *Hydrophilus* and *Hydrochara* are not sister genera (Toussaint *et al.*, 2017), which indicates a possibly more complex karyotype evolution in this lineage; the karyotype of *Hydrobiomorpha* Blackburn, 1888 has to be obtained to understand it properly. In contrast to the aquatic species, the two subfamilies with mainly terrestrial taxa, the Cyclominae and Sphaeridiinae, show a tendency for an increase in chromosome numbers, with diploid numbers ranging from 22 to 32. Based on data accumulated at the moment, it seems that the chromosome number is stable in the Coelostomatini ($2n = 26 + Xy$) and the Sphaeridiini ($2n = 22 + Xy$). Between-genera differences are present in the Cyclominae, Omicrini and Megasternini.

Within each genus, the chromosomes are often similar and the species differ by the size and darkness of C-bands, as we can see in *Laccobius*. *Enochrus* is another genus in which the chromosomes of the different species are often similar; however, the situation is more complicated as shown by striking chromosome differences between populations of *E. fuscipennis,* allowing even for detecting the heterozygous specimens in some cases.

The genus *Anacaena* appears to be the most chromosomally diverse of all Hydrophilidae, with diploid numbers ranging from 12–18, sex chromosomes including Xy_p and neo-XY systems, as well as parthenogenesis in *A. lutescens*. The initial stage of parthenogenesis in this species is associated with heterozygous deletion of a small terminal section of autosome 8. Subsequently, some populations developed triploidy (Shaarawi & Angus, 1991b). All *Anacaena* with known karyotypes are European species that are similar in morphology and likely closely related (Van Berge Henegouwen, 1986). *Anacaena* is much more diverse outside of Europe in terms of morphology and species numbers (Komarek, 2004, 2005, 2006, 2007, 2012). It would be interesting to investigate these non-European species to figure out whether the increased chromosomal diversity is the case for the whole genus. The only other member of the Anacaenini with a known karyotype is the New Zealand *Horelophus* with $2n = 16 + Xy$ as in other aquatic Hydrophilidae.

Cercyon is the only other genus with known variation in the number of chromosomes between species: we revealed species with the diploid number of 24 and 30 chromosomes. These differences may correspond to the earlier findings of possible polyphyly of the genus (Short & Fikáček, 2013) and indicate that the current concept of this genus needs a thorough revision.

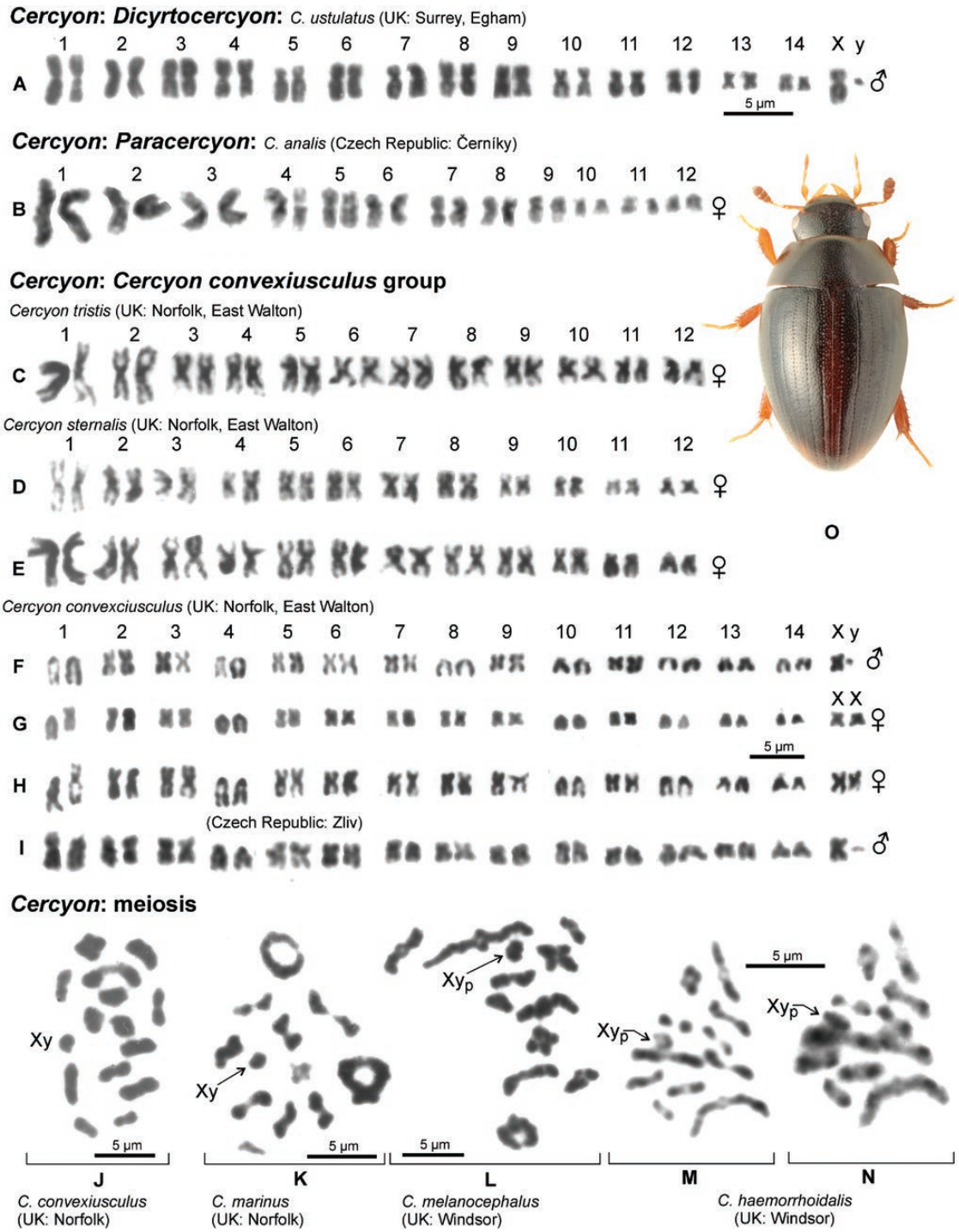

Figure 16. Karyotypes of the Megasternini: *Cercyon*. A–I, mitosis from midgut: (A) *Cercyon* (*Dicyrtocercyon*) *ustulatus*; (B) *Cercyon* (*Paracercyon*) *analis*; (C) *Cercyon tristis*; (D–E) *Cercyon sternalis*; (F–I) *Cercyon convexiusculus* (G–H, female, karyotypes from the same specimen showing variation in the form of chromosome 1). J–N, meiotic metaphase I from testes: (J) *Cercyon convexiusculus*; (K) *Cercyon marinus*; (L) *Cercyon melanocephalus*; (M–N) *Cercyon haemorrhoidalis*. A–M, without treatment. N, C-banded. O, habitus of *Cercyon analis*, from Fikáček (2019).

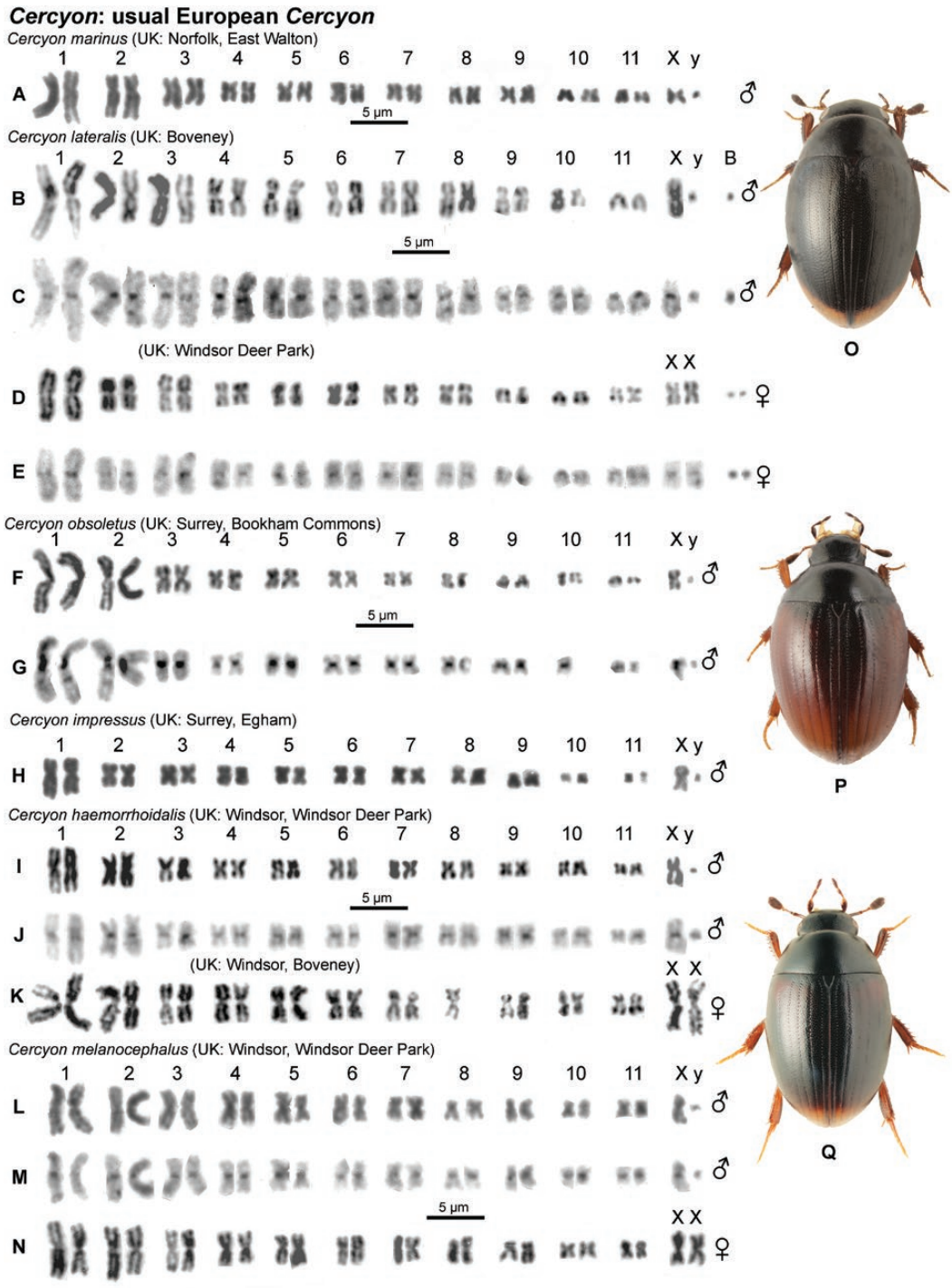

Figure 17. Mitotic karyotypes of *Cercyon* from midgut. (A) *Cercyon marinus*; (B–E) *Cercyon lateralis*; (F–G) *Cercyon obsoletus*; (H) *Cercyon impressus*; (I–K) *Cercyon haemorrhoidalis*; (L–N) *Cercyon melanocephalus*. A, B, D, F, H, I, K, L, N, without treatment. C, E, G, J, M, C-banded. Habitus figures: (O) *Cercyon marinus*; (P) *Cercyon impressus*; (Q) *Cercyon haemorrhoidalis*, from Fikáček (2019).

Table 1. Summary of known cytogenetic data for the family Hydrophilidae

Species	Reproduction mode	2*n*	Meioformula	Reference
Hydrophilinae: Amphiopini				
Amphiops mater	Sexual	18	–	This paper
Hydrophilinae: Berosini				
Berosus (s.s.) *affinis*	Sexual	18	$8 + Xy_p$	Shaarawi & Angus (1991a), Angus *et al.* (1994)
Berosus (*Enoplurus*) *fulvus*	Sexual	18	$8 + Xy_p$	Shaarawi & Angus (1991a)
Berosus (s.s.) *luridus*	Sexual	18	$8 + Xy_p$	Shaarawi & Angus (1991a)
Berosus (*Enoplurus*) *bispina*	Sexual	18	$8 + Xy_p$	Shaarawi & Angus (1991a)
Berosus (s.s.) *signaticollis*	Sexual	20	$9 + Xy_p$	Shaarawi & Angus (1991a)
Berosus (s.s.) *hispanicus*	Sexual	18	$8 + Xy_p$	Angus *et al.* (1994)
Berosus (*Enoplurus*) *indicus*	Sexual	18	$8 + Xy_p$	Agarawal (1960b)
Hydrophilinae: Laccobiini				
Laccobius (s.s.) *minutus*	Sexual	18	–	Angus & Shaarawi (1997)
Laccobius (s.s.) *colon*	Sexual	18	$8 + Xy_p$	Angus & Shaarawi (1997) (as *L. biguttatus*)
Laccobius (*Dimorpholaccobius*) *bipunctatus*	Sexual	18	$8 + Xy_p$	Angus & Shaarawi (1997)
Laccobius (*Dimorpholaccobius*) *ytenensis*	Sexual	18	$8 + Xy_p$	Angus & Shaarawi (1997) (as *L. atratus*)
Laccobius (*Dimorpholaccobius*) *striatulus*	Sexual	18	–	Angus & Shaarawi (1997)
Laccobius (*Dimorpholaccobius*) *sinuatus*	Sexual	18	–	Angus & Shaarawi (1997)
Laccobius (*Compsolaccobius*) *decorus*	Sexual	18	8 + Xy	This paper
Paracymus aeneus	Sexual	18	$8 + Xy_p$	This paper
Paracymus scutellaris	Sexual	18	$8 + Xy_p$	This paper
Tormus helmsi	Sexual	18	–	This paper
Tormus posticalis	Sexual	18	8 + Xy	This paper
Hydrophilinae: Hydrobiusini				
Hydrobius fuscipes	Sexual	18	$8 + Xy_p$	Smith (1960), this paper
Hydrobius arcticus	Sexual	18	8 + Xy	This paper
Hydrobius pui	Sexual	18	8 + Xy	This paper
Hydrobius rottenbergii	Sexual	18	8 + Xy	This paper
Hydrobius subrotundus	Sexual	18	8 + Xy	This paper
Limnohydrobius convexus	Sexual	18	8 + Xy	This paper
Limnoxenus niger	Sexual	18	$8 + Xy_p$	This paper
Hydrophilinae: Hydrophilini				
Sternolophus rufipes	Sexual	18	$8 + Xy_p$	Agarwal (1960a)
Sternolophus solieri	Sexual	18	8 + Xy	This paper
Tropisternus lateralis	Sexual	18	$8 + Xy_p$	Smith (1953, 1960)
Tropisternus mutatus sapucay	Sexual	18	$8 + Xy_p$	Pine *et al.* (2013)
Hydrochara caraboides	Sexual	30	14 + Xy	This paper

Table 1. Continued

Species	Reproduction mode	2*n*	Meioformula	Reference
Hydrochara flavipes	Sexual	30	14 + Xy_p	This paper
Hydrophilus acuminatus	Sexual	30	14 + Xy	Asana *et al.* (1942)
Hydrophilus indicus	Sexual	30	14 + Xy_p	Agarawal (1960a)
Hydrophilus piceus	Sexual	30	14 + Xy_p	Arnold (1909), this paper
Hydrophilus pistaceus	Sexual	30	14 + Xy	This paper
Hydrophilus triangularis	Sexual	30	14 + Xy_p	Smith (1953)
Chaetarthriinae: Chaetarthriini				
Chaetarthria simillima	Sexual	18	8 + Xy_p	Angus & Shaarawi (1997) (as *C. seminulum*), this paper
Chaetarthriinae: Anacaenini				
Anacaena bipustulata	Sexual	18	8 + Xy_p	Shaarawi & Angus (1991b)
Anacaena globulus	Sexual	16	7 + Xy_p	Shaarawi & Angus (1991b)
Anacaena limbata	Sexual	18	8 + Xy_p	Shaarawi & Angus (1991b)
Anacaena lutescens	Sexual	18	8 + Xy_p	Shaarawi & Angus (1991b)
	Parthenogenetic	18	–	Shaarawi & Angus (1991b)
	Parthenogenetic	3*n* = 27	–	Shaarawi & Angus (1991b)
Anacaena rufipes	Sexual	12	5 + NeoXY	Shaarawi & Angus (1991b)
Anacaena gaetanae	Sexual	18	8 + Xy	This paper
Horelophus walkeri	Sexual	18	8 + Xy	This paper
Enochrinae				
Cymbiodyta marginella	Sexual	18	8 + Xy	This paper
Cymbiodyta bifida	Sexual	18	8 + Xy_p	Smith (1960) (as *Helocombus bifidus*)
Enochrus s.s. *melanocephalus*	Sexual	18	8 + Xy	This paper
Enochrus (*Methydrus*) *affinis*	Sexual	18	8 + Xy	This paper
Enochrus (*Methydrus*) *coarctatus*	Sexual	18	8 + Xy	This paper
Enochrus (*Methydrus*) *nigritus*	Sexual	18	8 + Xy	This paper
Enochrus (*Methydrus*) *morenae*	Sexual	18	8 + Xy	This paper
Enochrus (*Methydrus*) *sauteri*	Sexual	18	8 + Xy	This paper
Enochrus (*Lumetus*) *bicolor*	Sexual	18	8 + Xy	This paper
Enochrus (*Lumetus*) *fuscipennis*	Sexual	18	8 + Xy_p	This paper
Enochrus (*Lumetus*) *halophilus*	Sexual	18	8 + Xy_p	This paper
Enochrus (*Lumetus*) *ochropterus*	Sexual	18	8 + Xy	This paper
Enochrus (*Lumetus*) *quadripunctatus*	Sexual	18	8 + Xy_p	This paper
Enochrus (*Lumetus*) *testaceus*	Sexual	18	8 + Xy	This paper
Acidocerinae				
Agraphydrus decipiens	Sexual	18	8 + Xy	This paper
Agraphydrus variabilis	Sexual	18	–	This paper
Helochares (s.s.) *lividus*	Sexual	18	8 + Xy_p	This paper
Helochares (s.s.) *obscurus*	Sexual	18	8 + Xy	This paper
Helochares (s.s.) *punctatus*	Sexual	18	8 + Xy_p	This paper

Table 1. Continued

Species	Reproduction mode	2*n*	Meioformula	Reference
Helochares (*Hydrobaticus*) *sauteri*	Sexual	18	8 + Xy	This paper
Cylominae				
Adolopus sp. 1	Sexual	24	11 + Xy	This paper
Adolopus sp. 2	Sexual	24	11 + Xy	This paper
Cyloma guttulatus	Sexual	24	–	This paper
Cyloma sp.	Sexual	24	11 + Xy	This paper
Cylomissus glabratus	Sexual	24	11 + Xy	This paper
Exydrus gibbosus	Sexual	30	–	This paper
Rygmodus modestus	Sexual	28	13 + Xy	This paper
Tormissus magnulus	Sexual	28	13 + Xy	This paper
Sphaeridiinae: Omicrini				
Noteropagus sp.	Sexual	22	–	This paper
Omicrogiton insularis	Sexual	26	12 + Xy	This paper
Paromicrus sp.	Sexual	26	–	This paper
Sphaeridiinae: Coelostomatini				
Coelostoma (s.s.) *orbiculare*	Sexual	28	13 + Xy	This paper
Dactylosternum flavicorne	Sexual	28	13 + Xy	This paper
Dactylosternum corbetti	Sexual	28	13 + Xy	This paper
Sphaeridiinae: Protosternini				
Protosternum abnormale	Sexual	32	–	This paper
Sphaeridiinae: Sphaeridiini				
Sphaeridium bipustulatum	Sexual	24	11 + Xy	Angus *et al.* (2000), this paper
Sphaeridium lunatum	Sexual	24	11 + Xy_p	Angus *et al.* (2000), this paper
Sphaeridium marginatum	Sexual	24	11 + Xy_p	Angus *et al.* (2000)
Sphaeridium scarabaeoides	Sexual	24	11 + Xy_p	Angus *et al.* (2000), this paper
Sphaeridiinae: Megasternini				
Cercyon (*Dicyrtocercyon*) *ustulatus*	Sexual	30	14 + Xy	This paper
Cercyon (*Paracercyon*) *analis*	Sexual	24	–	This paper
Cercyon (s.s.) *convexiusculus*	Sexual	30	14 + Xy_p	This paper
Cercyon (s.s.) *sternalis*	Sexual	24	–	This paper
Cercyon (s.s.) *tristis*	Sexual	24	–	This paper
Cercyon (s.s.) *haemorrhoidalis*	Sexual	24	11 + Xy_p	This paper
Cercyon (s.s.) *impressus*	Sexual	24	11 + Xy	This paper
Cercyon (s.s.) *lateralis*	Sexual	24	11 + Xy	This paper
Cercyon (s.s.) *marinus*	Sexual	24	11 + Xy_p	This paper
Cercyon (s.s.) *melanocephalus*	Sexual	24	11 + Xy_p	This paper
Cercyon (s.s.) *obsoletus*	Sexual	24	11 + Xy	This paper

Comparison with the Helophoridae

The Helophoridae are the only other family of the Hydrophiloidea with karyotypes known for a wider spectrum of species (Angus, 1982, 1983, 1986, 1989, 1992, 1996, 2015; Angus & Díaz Pazos, 1990; Angus *et al.*, 2005, 2016; Angus & Aouad, 2009; Angus & Toledo, 2010; Angus & Jia, 2020). The family comprises a single genus *Helophorus* divided into ten subgenera. There are two basic karyotype numbers, $2n = 16 + Xy_p$ (three subgenera, 15 karyotyped species) and $2n = 20 + Xy_p$ (four subgenera, 41 karyotyped species). The remaining three subgenera are unknown cytogenetically. Parthenogenesis is so far known in two species, both of which show triploidy (Angus & Jia, 2020).

Within both karyotype groups (with 18 and 22 chromosomes), there are complexes of species which are morphologically similar and hence difficult to tell apart but can be distinguished by their chromosomes. In the subgenus *Helophorus* s. str., Angus (1982) showed that *H. aquaticus* and *H. aequalis* had apparent differences in chromosome lengths, the position of the centromere, the amount of heterochromatin seen after C-banding and the size of the X chromosome. Similar differences were found in other cryptic species in this subgenus (Angus, 1989; Angus & Toledo, 2010). Interestingly, the chromosomes of *H. aequalis* and *H. grandis* are similar, which contrasts with the morphology of these species. Preliminary results of the DNA analysis (Fikáček *et al.*, unpubl.) surprisingly reveal both latter species as sister taxa, indicating that the chromosome morphology may follow the phylogenetic relationships better than morphology in this lineage. Chromosomes are more similar among the members of the species complexes in the subgenus *Rhopalohelophorus*. Still, clear differences between species can be found in the position of the centromere of some chromosomes and the size of the X chromosome [*H. minutus* complex (Angus, 1986, 1988); *H. flavipes* complex; (Angus, 1996)]. In all these cases, the chromosomal differences among closely related species are present and more profound than we observed in most representatives of the Hydrophilidae studied in this paper. In this aspect, the hydrophilid karyotypes can be considered as more conservative at species level than those of the Helophoridae.

CONCLUSIONS

In this study, we summarize the karyotype data of 95 species of the Hydrophilidae, covering all major phylogenetic lineages of the family by at least a single karyotyped representative. These results indicate that the karyotypes are conservative in most aquatic groups and more diverse in terrestrial clades. Further research, combined with analysis of genome size and with methods able to clarify homologies among groups with different chromosome numbers, is required to understand the evolution of the genome structure in the superfamily. It is also necessary to accumulate basic karyotype data for: (1) the genera that have not been karyotyped so far to understand karyotype evolution at the genus level; (2) additional species in genera for which one or two species have been karyotyped to confirm the stable chromosome number; (3) multiple representatives of the remaining hydrophiloid families (the Epimetopidae, Georissidae, Hydrochidae and Spercheidae) to understand karyotype evolution at the family level. Effort should be especially focused on the Cyclominae and Sphaeridiinae, in which we observed significant differences in karyotypes. A more detailed study of karyotype evolution within the giant water scavenger beetles (tribe Hydrophilini) and the *Anacaena* clade (tribe Anacaenini) would also be interesting. A detailed analysis of the mechanisms by which parthenogenetic populations appear in European *Anacaena lutescens* is also necessary.

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CONFLICT OF INTEREST

None of the authors declare any conflict of interest for this study.

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