Koleopterologische Rundschau	68	189 - 196	Wien, Juni 1998

Helophorus pallidipennis MULSANT & WACHANRU and *H. kervillei* d'ORCHYMONT as good species (Coleoptera: Helophoridae)

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Abstract

Chromosomal analysis of *Helophorus pallidipennis* MULSANT & WACHANRU (Coleoptera: Helophoridae) from Cyprus and Crete has revealed a karyotype the same as that obtained from Israeli material, and unlike that of Corfu material referred to *H. pallidipennis* var. *kervillei* d'ORCHYMONT. *Helophorus kervillei* is therefore regarded as a separate species. Laboratory rearing has shown that Cretan *H. pallidipennis* has three larval instars, as do most *Helophorus*, in contrast to Corfu *H. kervillei* which has already been shown to have only two instars. Characters enabling the two species to be distinguished, as both adults and final instar larvae, are given and illustrated. Adults are separable on the forms of the aedeagophore and pronotum. Final instar *H. kervillei* and second instar *H. pallidipennis* larvae appear to be separable on the form of the nasale. The urogomphal character used by ANGUS (1992) to separate second and third instar larvae is of doubtful value in *H. pallidipennis*. Third instar *H. pallidipennis* larvae are described in the context of the key to *Helophorus* larvae given by ANGUS (1992).

Key words: Coleoptera, Hydrophiloidea, Helophoridae, *Helophorus*, chromosomes, larvae.

Introduction

Helophorus pallidipennis, originally described by MULSANT & WACHANRU (1852) from "Karaman" in southern Anatolia (Turkey) is, as noted by ANGUS (1988), a species about which there has been considerable taxonomic confusion, not least because d'ORCHYMONT (1924, 1932) included the western Mediterranean *H. asturiensis* KUWERT in his concept of *H. pallidipennis*. ANGUS (1988) showed that the only major variation within eastern Mediterranean *H. pallidipennis* was associated with d'ORCHYMONT's var. *kervillei*, from near Ankara (Turkey), but that the main distinguishing feature of var. *kervillei*, the more slender aedeagophore with relatively longer parameres, appeared to be linked to normal *H. pallidipennis* by specimens with what appeared to be intermediate aedeagophores, with the full range of aedeagal variation represented in material from near Ankara. Later, ANGUS (1989) figured the chromosomes of *H. pallidipennis* from Corfu (Greece).

ANGUS (1992) described and keyed the larva of Corfu *H. pallidipennis*, showing that this species, like *H. kirgisicus* KNISCH, had only two larval instars, instead of the usual three. ANGUS (1988) pointed out that all Corfu *H. pallidipennis* were referable to var. *kervillei*, which tends to be smaller than the typical form, so that the larval information is incomplete. In the same work a note of possible taxonomic difficulties was mentioned in that Israeli "*pallidipennis*" was stated to have different chromosomes from Corfu material, though it was also reported, erroneously, to have only two larval instars.

Although ANGUS (1992) took the view that the Israeli species was apparently undescribed, there remained the possibility that the Israeli species was in fact *H. pallidipennis* while the Corfu one was *H. kervillei*. It was therefore essential to get chromosomes from "typical" *H. pallidipennis* from some of its European localities, or from Anatolia. To this end living material was obtained from both Cyprus (leg. Keith Miller) and Crete (collected by the author), and chromosomes from

both localities were found to be the same as those from Israel. A further, completely unexpected, discovery was that Cretan material has three larval instars, necessitating a re-evaluation of the Israeli material.

Chromosomes

Details of the material used for chromosomal analysis are given in Table 1. The methods used are described by ANGUS (1982) and SHAARAWI & ANGUS (1991). For preparations from mid-gut and testis the beetles were treated for 12.5 min. in both colchicine and hypotonic KCl, while for embryos these times were increased to 15 min.

Species	Locality of origin	Tissues used
H. pallidipennis	Cyprus: near Limassol	Embryo, mid-gut, testis
	Greece: Crete, Rethymnon	Embryo, mid-gut, testis
	Israel: Golan	Mid-gut
H. kervillei	Greece: Corfu, near Liapades	Embryo

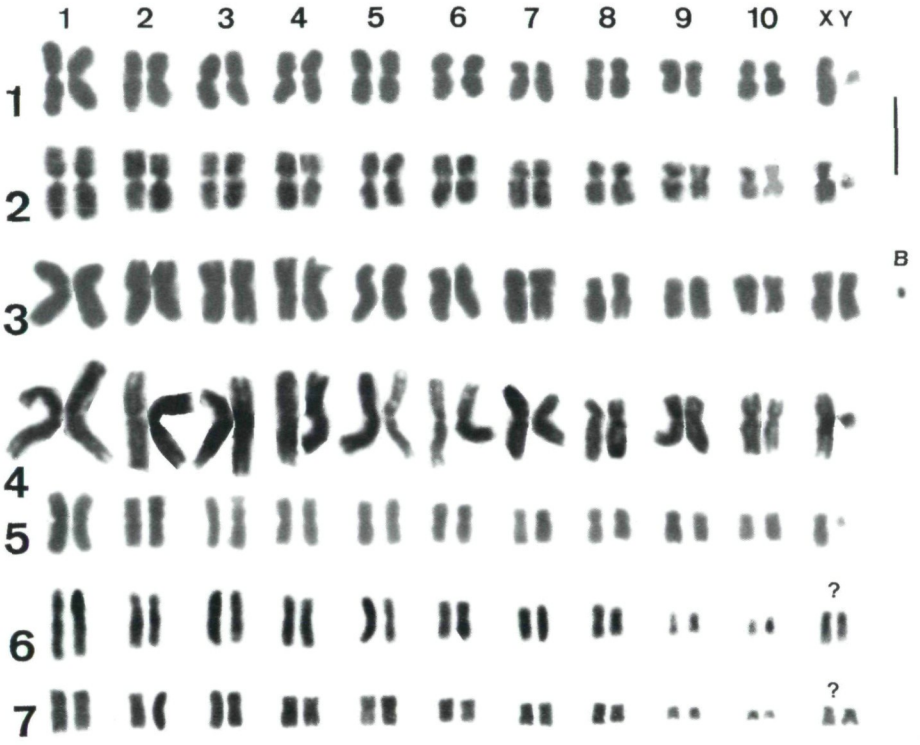
Table 1. Material used for chromosomal analysis.

Mitotic chromosomes are shown in Figs. 1 - 7. *H. pallidipennis* (Figs. 1 - 5) has 10 pairs of autosomes plus sex chromosomes which are XX (female) and XY (male), with the Y-chromosome dot-like and presumably forming a "parachute" association with the X at meiosis (Xy*p*), though this has not been confirmed. The relative chromosome lengths (R.C.L.) range from about 14 for autosome 1 to about 7 for autosome 10. (For a discussion of the use of R.C.L. see ANGUS & SHAARAWI 1997). The X-chromosome, with a R.C.L. of about 9, is similar in size to autosome pair 6. Most of the autosomes are more or less metacentric, but pairs 2, 7, 8 and 10 are submetacentric, with the short arm about half the length of the long arm, and this is also true of the X-chromosome.

The karyotypes appear the same in all the populations studied, the only variation detected being the presence of a B-chromosome in one of the Israeli specimens (Fig. 3).

The preparations of *H. kervillei* chromosomes (Figs. 6, 7), though rather poor and only of females, are very clearly distinct from those of *H. pallidipennis* because of the two pairs of conspicuously small chromosomes. Comparison of Figs. 5 - 7, which have the chromosomes of more or less the same range of absolute lengths, makes this difference very clear.

As in *H. pallidipennis*, there are 11 pairs of chromosomes, but because only females are available it is not possible to identify the sex chromosomes, and hence it is technically impossible to calculate R.C.L. as this requires calculation of the "total haploid autosome length" within the nucleus. As a provisional, tentative, solution to this problem, I have suggested as X-chromosome a pair of chromosomes apparently similar in size and shape to the X-chromosomes of *H. pallidipennis*. In view of the similarity of the X-chromosomes of many *Helophorus* (*Rhopalhelophorus*) species (the *H. minutus*-pattern of ANGUS 1989), this has a fair chance of being correct, and it facilitates comparison of the karyotypes of the two species. Using this arrangement, the R.C.L. of the longest autosomes (pair 1) is about 16, while those of the two shortest pairs are about 5 and about 3. The pair chosen as X has a R.C.L. of about 7.5, similar to autosome pair 8. Autosome pair 6, which in *H. pallidipennis* is most similar in length to the X-chromosome, has a R.C.L. of about 9, while the R.C.L. values for pairs 7 and 9 are about 8.4 and about 5. Most of the chromosomes are submetacentric, with one arm clearly somewhat shorter than the other, with only pairs 3, 5, 6 and 8 appearing almost metacentric.



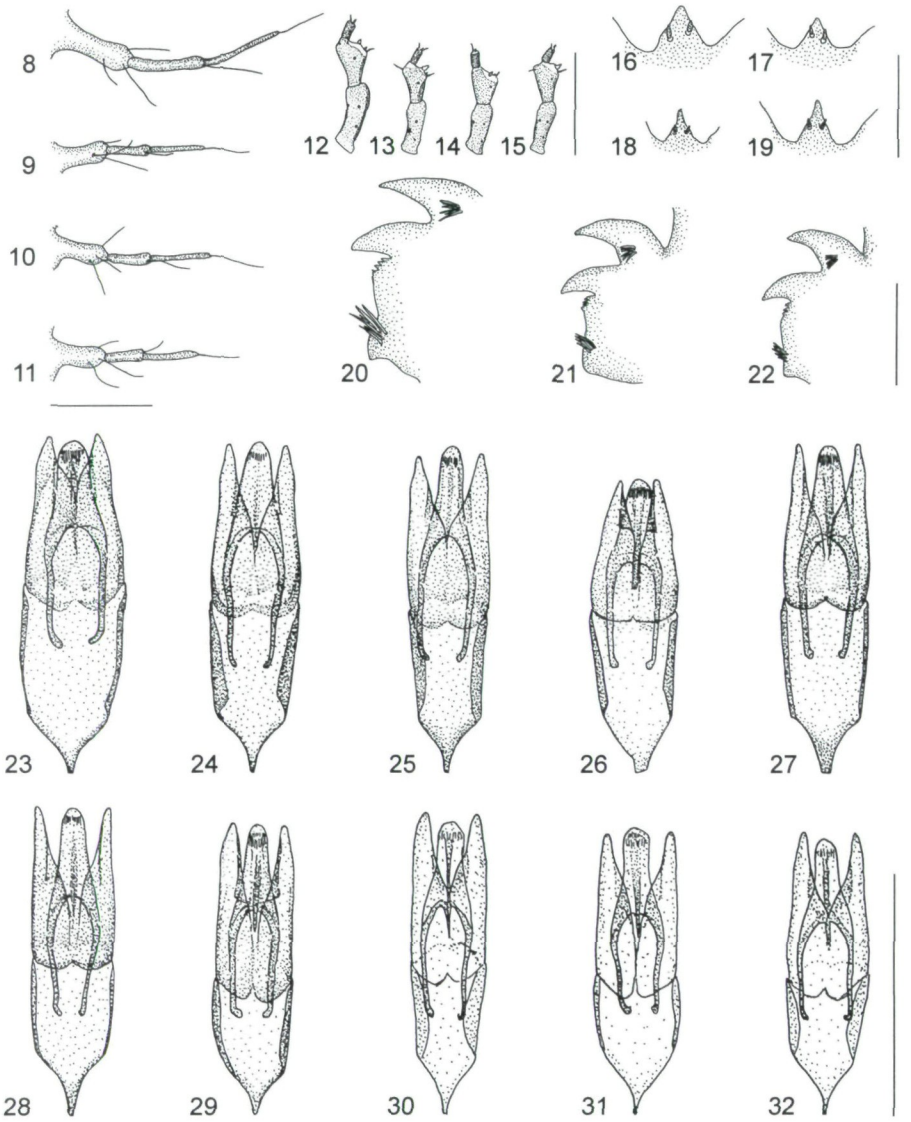
Figs. 1 - 7: Mitotic chromosomes. The scale line represents 5 μm. 1) *Helophorus pallidipennis,* male, testis, Cyprus; 2) *H. pallidipennis*, male, testis, Crete; 3) *H. pallidipennis*, female, mid-gut, Israel; 4) *H. pallidipennis*, male, mid-gut, Israel; 5) *H. pallidipennis*, male, embryo, Cyprus; 6, 7) *H. kervillei*, female, embryo, Corfu.

Although there is clearly a need for better material, especially male karyotypes, of *H. kervillei*, it is clear that the karyotypes of the two species are different, confirming their status as separate species.

Immature stages

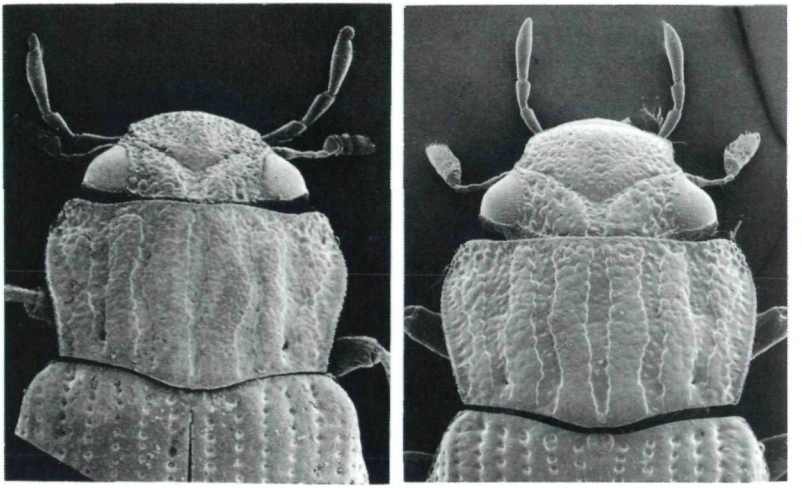
H. kervillei. The egg cocoon and larva are described (as *H. pallidipennis*) by ANGUS (1992). The egg cocoon is a Type 3 cocoon as categorised in that work, with a trailing thread-like mast formed from the back of the tube which tops the egg bag in other types of cocoon. The cocoon is similar to those of *H. flavipes* F. and *H. seidlitzii* KUWERT (ANGUS 1992, Fig. 35 h, i).

As mentioned in the introduction, *H. kervillei* has only two larval instars. This was confirmed by keeping a second stage larva in its rearing box until it pupated, and I have the exuviae of this larva, and the cleared pupa, mounted on a microscope slide. The final instar larvae were considered by ANGUS (1992) to be equivalent to normal third instar larvae rather than to second instar ones which would moult into third instar before pupating. This was on the basis of the more elongate second segments of the urogomphi (Fig. 9), which are about 5.5 times as long as wide about one third of the way from the base.



Figs. 8 - 22: Larval structures, traced from photographs. 8 - 11: urogomphi. 8) *Helophorus pallidipennis*, 3rd instar, Crete; 9) *H. kervillei*, 2nd (final) instar, Corfu; 10) *H. pallidipennis*, 2nd instar, Israel; 11) *H. pallidipennis*, 2nd instar, Crete; 12 - 15: antennae. 12) *H. pallidipennis*, 3rd instar, Crete; 13) *H. kervillei*, 2nd (final) instar, Corfu; 14) *H. pallidipennis*, 2nd instar, Crete; 15) *H. pallidipennis*, 2nd instar, Israel; 16 - 19: nasales. 16) *H. pallidipennis*, 3rd instar, Crete; 17) *H. kervillei*, 2nd (final) instar, Corfu; 18) *H. pallidipennis*, 2nd instar, Crete; 19) *H. pallidipennis*, 2nd instar, Israel; 20 - 22: mandibular bases. 20) *H. pallidipennis*, 3rd instar, Crete; 21) *H. kervillei*, 2nd (final) instar, Corfu; 22) *H. pallidipennis*, 2nd instar, Crete. The scale line represents 0.5 mm for Figs. 8 - 11, 0.3 mm for Figs. 12 - 15, and 0.1 mm for Figs. 16 - 22.

Figs. 23 - 32: Aedeagophores, traced from photographs. The scale line represents 0.5 mm. 23 - 28: *Helophorus pallidipennis*. 23 - 25) from the Ankara region, leg. Gadeau de Kerville; 26) *H. vinctus*, holotype; 27, 28) from Israel; 29 - 32: *H. kervillei*; 29) *H. kervillei*, lectotype; 30 - 32) from Corfu. Figs. 23 - 29 drawn by Sarah Wroot.



33 34

Figs. 33, 34; Scanning electron micrographs of heads and pronota. The scale line represents 1 mm. 33) *Helophorus pallidipennis* (*H. reitteri*, lectotype); 34) *H. kervillei* from Corfu.

As given in the key by ANGUS (1992), second instar larvae have the second urogomphal segment 2.5 - 4.0 times as long as wide, as against 5 - 8 times as long in third instar larvae. This can be a rather fine distinction, not helped by a tendency of lab-reared larvae to fail to moult their urogomphi cleanly, giving rumpled segments. Nevertheless, of my four Corfu larvae (including the exuviae already mentioned), it is possible to get approximate values of between 5.5:1 and 6:1 from three larvae (five urogomphi). In one larva the urogomphi are clearly hopelessly crumpled, and in two cases the length of the second segment has been estimated from that of segment three. Segment two is about 0.8 times as long as segment three.

As noted by ANGUS (1992), the *H. kervillei* larvae would run to the loosely defined "*H. minutus*-group" with their combination of moderately developed basal mandibular hair tufts (Fig. 21) and nasales without teeth on the underside (Fig. 17). The abdominal sclerites may be very indistinctly darkened, but may be bolder, but the most characteristic feature is the feeble darkening of the head capsule round the ocelli - not obviously darker than the apical segment of the antennae. Fully fed larvae are about 5.5 - 6.2 mm long (urogomphi excluded), and their head capsules (at eye level) are 0.45 - 0.55 mm wide, with the distance between the antennal insertions 0.28 - 0.35 mm. The nasale has the form of a straight-sided equilateral triangle, though slightly narrower in one specimen.

H. pallidipennis. Egg cocoons have been obtained from populations from Crete, Cyprus and Israel. They appear identical with those of Corfu *H. kervillei*.

Larvae have been obtained from Cretan and Israeli beetles. None of the Israeli material was taken beyond second instar. Pne good larva was used for (largely unsuccessful) chromosome work and the second one was used for a cleared preparation. At the time, these larvae were thought to be final instar because of their similarity to Corfu material. Subsequent information obtained from Cretan material shows this assumption to be unjustified, and there is now no reason to regard the Israeli material as departing from the normal three-instar larval development typical of *Helophorus*. The Cretan material, obtained in April 1996, comprises four larvae. Two were mounted as cleared preparations in balsam as second instar, while the others were kept alive in anticipation of their pupation, to confirm that *H. pallidipennis*, like *H. kervillei*, had only two larval instars. This led to the unexpected discovery of third instar larvae, and these two specimens are now mounted in balsam.

The third instar larvae are just over 7 mm long, and have head widths of 0.60 and 0.75 mm, with the distances between the antennal insertions 0.46 and 0.50 mm. The urogomphi (Fig. 8) have the second segment about 7 times as long as wide. The nasale (Fig. 16) lacks ventral teeth and is a more or less equilateral triangle, though with the sides tending to bulge outwards medially. The basal mandibular hair tuft (Fig. 20) is large, and the antennae (Fig. 12) are fairly robust. The post-pronotal band (in the membrane between the pro- and mesonota) is very dark, but the abdominal sclerites are very pale, with only tergite 9 distinctly darkened. This is a rather distinctive larva. In the key given by ANGUS (1992) it would fall in the loosely defined "*H. flavipes*-group", and as such it would run to couplet 40, then to 42 because of its large size. The abdominal spiracles are moderately large, as in *H. obscurus* MULSANT (ANGUS 1992, Fig. 39 l), which would run the larvae to couplet 43. At this stage the larvae are distinctive because of the very dark post-pronotal bands (ANGUS 1992, Fig. 39, q, r) and pale abdomens.

The second instar larvae are more of a problem. Their general appearance is similar to final instar Corfu *H. kervillei*, though the second segment of the urogomphi is in fact less elongate. Israeli material (Fig. 10), regarded by ANGUS (1992) as final instar, but now regarded as normal second instar, has the segment about 4.8 times as long as wide, while the Cretan specimens (Fig. 11) have it about 3.8 times as long as wide. In view of the difficulties encountered in obtaining this measurement, the distinction between second and third instar larvae may not always hold. The second instar *H. pallidipennis* larvae resemble the final instar *H. kervillei* in their smallish basal mandibular hair tufts (Fig. 22) and antennal shape (Figs. 18, 19), and in having the head capsule rather feebly darkened round the ocelli. The abdominal tergites may be virtually invisible in cleared preparations, or may be distinct though pale. The nasales of both Cretan and Israeli material (Figs. 18, 19) are narrower than in *H. kervillei*, forming an isosceles triangle with the apex produced as a narrow projection beyond the median lateral bulges.

Taxonomy

H. pallidipennis MULSANT & WACHANRU, 1852

Helophorus pallidipennis MULSANT & WACHANRU 1852: 6. Type locality: "la Caramanie" (not "Carmanie" as given by ANGUS 1988) (The Karaman region refers to an extensive area of southern Anatolia north of the Taurus Mountains. The modern town of Karaman lies at the southern edge of this region. (M.A. Jäch, in litt. 9.x.97.)). Type not seen, not located. For discussion see ANGUS (1988).

Helophorus reitteri KUWERT 1885: 252. Type locality: Greece, Mt. Parnassos. Types seen and lectotype designated by ANGUS (1988). Syn. by ANGUS (1988).

Helophorus vinctus SHARP 1916: 167. Type locality: "Besica Bay" (W Anatolia, S of the entrance to the Dardanelles, near the archaeological site of ancient Troy). Holotype seen and figured by ANGUS (1969). Syn. by ANGUS (1988).

Additional material: Cyprus, Greece (Mt. Parnassos, Attica, Crete), Turkey: Anatolia ("Besica Bay"; Sirnak, SE Turkey (leg. Jäch); Karacadag near Diyarbakir (leg Jäch), Ankara "région d'Ankara" (leg. Gadeau de Kerville, in coll. d'Orchymont)); Israel (Golan & Gallilee, many specimens, leg. Jäch, Ortal and Israel Ecological Survey).

H. kervillei d'ORCHYMONT, 1932

Helophorus pallidipennis var. *kervillei* d'ORCHYMONT, 1932: 394. **stat.n.** Type locality: "Région d'Ankara", Turkey, leg. Gadeau de Kerville. Types seen and lectotype designated by ANGUS (1969).

Additional material: Corfu, coll. d'Orchymont and my collection, many specimens.

Although I have not attempted to assemble all possible material referable to these species, both

appear to have rather restricted distributions at the eastern end of the Mediterranean. It is worth noting that neither species is represented in the collections of the Zoological Institute, St Petersburg, so the distributions would appear not to extend either along the northern coast of the Black Sea or into the Transcaucasus. It is also worth noting that both species occur near Ankara, but that only *H. kervillei* is present on Corfu; further, all the other *H. pallidipennis* localities appear to lack *H. kervillei*. Although the two *H. kervillei* localities are widely separated, they are on about the same latitude and north of most of the *H. pallidipennis* localities.

Both species are normally pale yellowish brown, though occasionally the elytra may be darker. They are characterised by their 8-segmented antennae with only two small segments between the pedicel and the cupule; their pale pronota, yellow to pale brown with greenish bronze reflections, lightly sculptured over the disc and neither strongly cordiform nor conspicuously narrow compared with the elytra; and their elytral flanks (pseudepipleura) which are distinctly visible from below, about half the width of the epipleurs opposite the metasternum.

H. pallidipennis, 2.8 - 4.9 mm long, is on the whole a little larger than *H. kervillei* which on Corfu is 2.7 - 4.0 mm long, while ANGUS (1969) mentions a (Turkish) paralectotype of *H. kervillei* as being 4.2 mm long.

They are best separated by the forms of their pronota and aedeagophores, though both species vary in these characters. The aedeagophore of *H. pallidipennis* (Figs. 23 - 28), 0.6 - 0.7 mm long, is normally larger than that of *H. kervillei* (Figs. 29 - 32), which is 0.58 - 0.60 mm long. The parameres of *H. kervillei* are generally slender with their outer margins normally straight, parallel to one another, and the apices bluntly angled. However, there is some variation and Fig. 32 shows a specimen with less parallel paramere margins. The basal piece is normally noticeably short, but Fig. 31 shows a specimen in which it is nearly as long as the parameres. The parameres of *H. pallidipennis* are normally more robust than those of *H. kervillei*, with their outer margins neither largely straight nor parallel to one another. Israeli *H. pallidipennis* (Figs. 27, 28) have the parameres more attenuated apically and more sharply pointed. The basal piece appears to be relatively longer in *H. pallidipennis* than in *kervillei*, but there is overlap between the two species in this respect (compare Figs. 25 and 30).

The pronota are shown in Figs. 33 and 34. In *H. pallidipennis* (Fig. 33) the pronotal sides are generally straightened or slightly concave over their basal third, and the marginal groove is distinctly widened medially and its outer part tends to be markedly raised towards the narrow lateral margin, giving a somewhat flared appearance. In *H. kervillei* (Fig. 34) the pronotal sides are normally more evenly rounded to the base, though they may be somewhat straightened over the basal third, and the marginal grooves are less conspicuously widened and flared medially.

Acknowledgements

This sort of chromosomal investigation requires live material from a number of widely separated localities, for which help with the supply of livestock is almost essential. I am therefore very grateful to Dr. K. Miller (Cyprus) and Dr. R. Ortal (Hebrew University of Jerusalem) for sending living *H. pallidipennis* from Cyprus and Israel, as well as to Mr. R. Thompson (Belfast) who drew my attention to a prime collecting site on Crete. I thank the following for the opportunity to study museum material in their care: M.P. Dessart (I.R.S.N.B., Brussels) (d'Orchymont collection); Dr. O. Merkl (Termeszettudomanyi Muzeum, Budapest) (Reitter collection); Dr. M.A. Jäch (Naturhistorisches Museum, Vienna) (Israeli and Turkish material, as well as for information on various Turkish localities); Dr. R. Ortal (Jerusalem) (Israeli material); and Mr. M. Brendell (Natural History Museum, London) (Sharp collection). Finally, I thank the Electron Microscope Unit and the School of Biological Sciences, Royal Holloway, for the facilities to carry out this research.

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Chromosomes of *Chaetarthria* and *Laccobius* (Hydrophilidae)

In the paper by ANGUS & SHAARAWI (1997, Koleopterologische Rundschau 67: 181 186), the figures of the chromosomes were printed with such a high degree of contrast that much of the information was lost. They are reproduced here with less contrast, so that the details may be seen. The authors take this opportunity to thank the editor for providing these reprinted figures.

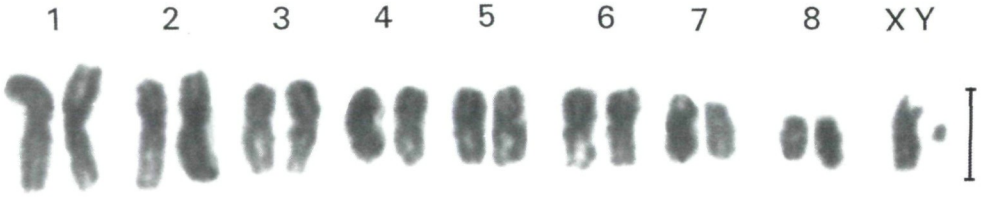

Fig. 1: *Chaetarthria seminulum*: mitotic chromosomes from male mid gut, Cothill, Oxfordshire. The scale line represents 5 μm.

Fig. 2, a - i *Laccobius* spp.: mitotic chromosomes. The scale line represents 5 μm. a) *L. minutus*, male embryo, Wraysbury, Berkshire; b) *L. minutus*, female embryo, C-banded, Wraysbury; c) *L. biguttatus*, male mid gut, Sheppey, Kent; d) *L. biguttatus*, female embryo, East Walton, Norfolk; e) *L. bipunctatus*, male mid gut, New Forest, Hampshire; f) *L. bipunctatus*, female embryo, Wraysbury, Berkshire; g) *L. bipunctatus*, female embryo, C-banded, Cothill, Oxfordshire; h) *L. atratus*, male mid gut, New Forest, Hampshire; i) *L. atratus*, male mid gut, C-banded, New Forest.

Fig. 3 (p. 198), a - i *Laccobius* chromosomes. The scale line represents 5 μm. a - f: mitotic chromosomes. a) *L. striatulus*, male embryo, Radley, Oxfordshire; b) *L. striatulus*, female embryo, C-banded, Radley; c) *L. sinuatus*, male embryo, Radley; d) *L. sinuatus*, female embryo, Radley; e) *L. sinuatus*, female embryo, C-banded, Radley; f) *L. sinuatus*, male embryo with 2 Y-chromosomes, Radley. g - i: meiotic chromosomes, first metaphase from testis; g) *L. biguttatus*, Sheppey, Kent; h) *L. bipunctatus*, New Forest, Hampshire; i) *L. atratus*, New Forest.