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A new, chromosomally distinct Italian *Helophorus* F. similar to *H. aequalis* THOMSON, and notes on some other species in Italy (Coleoptera: Helophoridae)

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Abstract

Chromosomal analysis shows that Italian material thought to belong to *Helophorus aequalis* THOMSON (Coleoptera: Helophoridae) is a new species clearly recognizable because of the larger nucleolus organizer bearing autosome (autosome 6). The new species, *H. thauma*, is described and is compared with *H. aequalis* and *H. aquaticus* (LINNAEUS). Comments on the distribution and genetic assessment of *H. flavipes* (FABRICIUS), *H. obscurus* MULSANT and *H. brevipalpis* BEDEL are given. *Helophorus strigifrons* THOMSON and *H. lapponicus* THOMSON are recorded for the first time from Italy.

Key words: Coleoptera, Helophoridae, Helophorus, chromosomes, new species, new records, Italy.

Introduction

Living *Helophorus* F. of the *H. aquaticus-aequalis* group were collected in the Parma region of northern Italy, with a view to providing a series of chromosomally identified reference specimens to facilitate identification of material by M.A. Toledo. However, inspection of the resulting preparations showed that the material could not be referred to either *H. aquaticus* or *H. aequalis*, but was in fact a hitherto unrecognised species, here described as *H. thauma*.

Material and methods

The material used for chromosome investigation is listed in Table 1.

The chromosome preparations in the present study were from mid-gut cells, using the methods described by SHAARAWI & ANGUS (1991) and ANGUS (1982). Preparations were photographed at $3000 \times$ and the images used to build the karyotypes. RCL (Relative Chromosome Length) was used to compare chromosome sizes in the karyotypes. Some of the earlier preparations illustrated were from developing embryos (ANGUS 1982).

The abbreviations for collections holding material discussed are as follows:

FPC Fernando Pederzani collection, Ravenna, Italy

MTC Mario Toledo collection, Parma, Italy

NHML The Natural History Museum, London, UK

SRC Saverio Rocchi collection, Firenze, Italy

Chromosome analysis

Mitotic chromosomes, arranged as karyotypes, are shown in Fig. 1a-l. The chromosomes of H. thauma (Fig. 1a-c) resemble those of H. aequalis (Fig. 1d-f) in the short autosomes 7 and 8, the acrocentric X chromosome and the fairly strong C-bands on all the autosomes, including pairs 1 and 2, all features clearly distinct from H. aquaticus (L.) (Fig. 1g-l). However, autosome 6, which in this group of *Helophorus* bears the secondary constriction (nucleolus organizer) (ANGUS 1982) is much longer than in H. aequalis, RCL more than in H. aquaticus. Because the karvotype of H. aquaticus is clearly very different from that of H. thauma, detailed RCL comparisons are not given here, but the values are given by ANGUS (1982). RCL comparisons of H. thauma and H. aequalis are given in Table 2. In addition to the H. aequalis data from ANGUS (1982), new data from Bavarian material (leg. M. Hess) are included as a control. These data are partly (50 %) from embryos, like the data in ANGUS (1982), but are partly from testis (25 %) and mid gut (25 %) of an adult male. In all cases the 95 % confidence intervals of these new data overlap those of the 1982 data, indicating a lack of significant difference between the values in the two data sets. However, when the *H. thauma* values are compared, not only is autosome 6 shown to be far larger than in *H. aequalis*, but autosomes 7 and 8 are shown to be clearly (and significantly) smaller. This degree of chromosome size difference must imply that there are important translocational differences (transfer of material between non-homologous chromosomes) between H. thauma and H. aequalis, which would preclude successful meiosis in any hybrids. This confirms that *H. thauma* is a distinct species, not a variant of *H. aequalis*.

Species	Locality	Number of specimens			
H. thauma	ITALY, Provincia di Parma, Torrile near Parma	$6 \sigma \sigma, 6 \varsigma \varsigma$ (holotype + 11 paratypes),			
	ITALY, Provincia di Parma, Parma	$2 \sigma \sigma, 1 q q$ (paratypes)			
H. aequalis THOMSON	FRANCE, Indre et Loire, Indre	ca 10 embryos (1982 data)			
	GERMANY, Bavaria, near Straubing	1 ♂, 2 embryos (♂, ♀)			
H. aquaticus (L.)	FRANCE, Puy de Dôme	2 embryos			
	GERMANY, Bavaria, near Straubing	13			
	ITALY, Abruzzo, Campo Felice	13			

Table 1: Material used for chromosome analysis and illustration.

Karyotypes of *H. aquaticus* are shown in Fig. 1g–l. Fig. 1g–h shows the embryo preparations illustrated by ANGUS (1982). Fig. 1h shows a karyotype from the mid-gut of a Bavarian male, with autosome 6 appearing slightly larger than autosomes 3–5, normal in gut at testis preparations, but less prominent in those from embryo. This feature is also shown by the preparation from mid-gut of an Italian male (from Campo Felice in the Abruzzi mountains) (Fig. 1i–j), and this preparation also shows the smaller C-bands of autosomes 1 and 2. Fig. 11 shows a

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further mid-gut preparation from the same male, in which the chromosomes are more condensed and autosome 6 now appears very much longer than pairs 3-5. This is a condensation effect and there is no reason to suppose that Italian *H. aquaticus* is different from material from other parts of its range.

Table 2:	Helophorus	thauma	and	Н.	aequalis,	Relative	Chromosome	Length,	mean,	95	%	confidence
intervals	by t-test, nur	nber of c	observ	ati	ons (N).							

Chromosome	H. aequalis (1982 data)	<i>H. aequalis</i> (Bavaria, 1995)	H. thauma		
1	20.88	20.65	20.81		
	20.47-21.29	20.29-21.02	20.26-21.36		
	N = 30	N = 24	N = 20		
2	17.31	17.03	16.66		
	16.97-17.66	16.73-17.32	16.17–17.14		
	N = 30	N = 24	N = 20		
3	12.10	12.39	12.10		
	11.86-12.34	12.17-12.61	11.87–12.32		
	N = 29	N = 24	N = 20		
4	11.38	11.67	11.47		
	11.18-11.57	11.47-11.88	11.31–11.63		
	N = 29	N = 24	N = 20		
5	11.15	11.12	10.95		
	10.88-11.42	10.91-11.32	10.75-11.15		
	N = 29	N = 24	N = 20		
6	9.44	9.10	13.39		
	9.01-9.86	8.79–9.41	12.96-13.82		
	N = 28	N = 24	N = 20		
7	9.52	9.81	8.49		
	9.22-9.82	9.52-10.10	8.13-8.85		
	N = 28	N = 24	N = 20		
8	8.19	8.35	6.20		
	8.00-8.37	8.12-8.58	5.94-6.46		
	N = 28	N = 24	N = 20		
Х	8.34	8.80	7.73		
	7.97-8.70	8.43-9.17	7.30-8.14		
	N = 20	N = 15	N = 15		

	1	2	3	4	5	6	7	8	Ху	
а	11	И	۲¢	88	XX	16	11	g X	۶.	
b	21	38	ž.X	K.Z	58	10	X N	**	ð ''	
с	Н	ĸ	83	88	44	ŧŧ.	••	••	9 **	
d	R	ł	f 5	5.8	88	88	15	**	Ľ	
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j	83)(* R	88	88	66	21	88	8 -	
k)3)<	11	ŧ,	Ř8	€¢	h	89	ŀ	
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Helophorus (s.str.) thauma sp.n.

Holotype: ♂, Italy, Provincia di Parma, Torrile. Grassy rain-pools. 5.v.2009. M.A. Toledo leg. Chromosome prep. 1, 14.v.2009. R.B. Angus. NHML.

Paratypes: $5 \sigma \sigma$, $6 \varphi \varphi$, same data as holotype. Chromosome preparations 2, 14.v.2009, 1–4, 17.v.2009, 1–6, 21.v.2009, R.B. Angus. $4 \sigma \sigma$, 1φ , Italy, Provincia di Parma, Parma University Campus. Rain-pools. 22.iv.2009. M.A. Toledo leg., chromosome preparations 1–3, 1.v.2009, R.B. Angus, with $2 \sigma \sigma$ not used for chromosome preparations. The paratypes are shared between NHML (R.B. Angus collection) and MTC.

Length: 4.7–5.6 mm (\$\vec{d}\$), 5.2–6.1 mm (\$\vec{d}\$); breadth: 2.1–2.4 mm (\$\vec{d}\$), 2.3–2.7 mm (\$\vec{d}\$).

Head: pitchy bronze to dull greenish bronze, granulate, the granulation tending to be weaker medially. Y-groove with stem either parallel sided or widened anteriorly, brighter bronze than rest of head. Maxillary palpi fairly short, yellow, apical segment asymmetrical, darkened at tip. Antennae brown, 9-segmented.

Pronotum: colour as head. Coarsely but not roughly granulate, the granulation sometimes reduced on the internal intervals. Grooves moderately wide and deep, submedians angled out medially (sometimes bluntly so), recurved a third of the way from each end. Floors of grooves crinkled, sometimes more shining bronze than intervals. Marginal grooves dull yellow.

Elytra: mainly dull brown, sutural Λ-mark made up of three black spots, the median one on the suture about half way down the elytra, and the lateral ones in interstice 2 slightly nearer the apex. There is a further spot on interstice 6, opposite the sutural one. Some specimens have the ground colour rather paper and may have indistinct paler mottling. Interstices moderately ridged, about twice the width of the striae. In some specimens interstices 2, 4 and 6 are slightly more raised than the others. Intercalary striae well developed. Flanks narrow, scarcely visible from below.

Legs: dull yellow, moderately long, tarsi with fairly weak swimming-hairs.

Abdomen (Fig. 3): sternite 7 finely crenulate, as in *H. aequalis* and *H. aquaticus*.

Aedeagophore (Fig. 2a–c): length 0.83–0.92 mm. Paramere and basal piece lengths almost equal. Outer margins of parameres more or less straight, sometimes weakly concave medially, weakly convex on apical sixth. If somewhat desiccated the outer margins may appear convex.

Etymology: *thauma* is a Greek word for an apparition and refers to the totally unexpected occurrence of this new species.

Discussion

The discovery of *H. thauma* came as a complete surprise and raises the question as to how many other species there may be in this group, and in particular, whether *H. aequalis* occurs at all in the Mediterranean lands. At this stage it seems very likely that all Italian records of *H. aequalis* refer to *H. thauma*. There do not appear to be any morphological characters which enable material of the two species to be referred to either one.

[◄] Fig. 1 (opposite page): Mitotic chromosomes of males of *Helophorus* spp., arranged as karyotypes. **a**, *H. thauma*, holotype, mid-gut nucleus, plain; **b**, **c**, *H. thauma*, paratype from Parma, mid-gut, **b** plain, **c** the same nucleus, C-banded; **d**, *H. aequalis*, embryo from France, C-banded; **e**, *H. aequalis*, mid-gut nucleus from Bavaria, plain; **f**, *H. aequalis*, embryo from France, plain; **g**, *H. aquaticus*, embryo from France, plain; **j**, *k*, *H. aquaticus* from Campo Felice, **j** plain, **k** the same nucleus C-banded; **l**, *H. aquaticus* from Campo Felice, mid-gut from the same beetle as **j**, **k**, but more contracted, plain.

The other question raised concerns those specimens of the *H. aquaticus-aequalis* group with slightly larger aedeagophores. ANGUS (1988) discusses specimens from western Turkey, and concludes that they are referable to *H. aquaticus*, and also mentions a specimen from Comana Vlasca (Romania) in Orchymont's collection. ANGUS (1992a) again refers to this material, and adds that there are a further two specimens, also in the Orchymont collection, from Kutaisi (Kutais-ça on the beetle label), Georgia. Some of these aedeagophores are shown in Fig. 2d–g. The Turkish material (Fig. 2e–f) seems clear *H. aquaticus*, with a long basal piece and the outer margins of the parameres convex. The Comana Vlasca specimen (Fig. 2d) may also be *H. aquaticus* as the basal piece is conspicuously long. However, the parameres are similar, in both size and shape, to those of the holotype of *H. thauma* (Fig. 2a), so the identification is uncertain. The Georgian material (Fig. 2g) is the largest of all, 1 mm long, and its form is definitely of the *H. aequalis* rather than the *H. aquaticus* pattern. Its identity is a mystery, probably only solvable if chromosomal data become available.



Fig. 2: Aedeagophores of *Helophorus* spp. **a**, *H. thauma*, holotype; **b**, *H. thauma*, paratype from Torrile with the outer margins of the parameres convex as a result of desiccation; **c**, *H. thauma*, paratype from Parma, the largest aedeagophore encountered; **d**, *H. ?aquaticus* from Comana Vlaca, Romania; **e–f**, *H. aquaticus* from Turkey; **g**, *H.* sp. from Kutaisi, Georgia. Scale = 1 mm.



Fig. 3: *Helophorus thauma*, paratype from Parma, abdominal apex showing the crenulations on sternite 7. Scale = 0.5 mm.

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Notes on other Helophorus species from Italy

Helophorus (Atracthelophorus) brevipalpis BEDEL, 1881

Material examined:

LOMBARDIA: $19 \ _{\varphi} \ _{\varphi}$ (MTC): Lodi prov., Somaglia, Monticchie WWF natural reserve, 7.iv.1999, leg. M. Toledo; $9 \ _{\varphi} \ _{\varphi}$ (MTC): Brescia prov., Iseo, Torbiere d'Iseo [bogs south of lake Iseo], 26.vi.1985, leg. M. Toledo; $5 \ _{\varphi} \ _{\varphi}$ (MTC): Cremona prov., Soncino, ditch nr. Cascina Busta, 25.vi.1983, leg. M. Toledo; $4 \ _{\varphi} \ _{\varphi}$ (MTC): Cremona prov., Gabbioneta, oxbow lake of river Oglio, 2.vi.1985, leg. M. Toledo; $3 \ _{\varphi} \ _{\varphi}$ (MTC): Mantova prov., Acquanegra sul Chiese, Le Bine WWF natural reserve, 6.vi.1996, leg. M. Toledo; $3 \ _{\varphi} \ _{\varphi}$ (MTC): same, 30.vi.1996, leg. M. Toledo; $9 \ _{\varphi} \ _{\varphi}$ (MTC): same, 23.–28.iv.1998, leg. M. Toledo; $4 \ _{\varphi} \ _{\varphi}$ (MTC): same, 4.v.2000, leg. M. Toledo; $6 \ _{\varphi} \ _{\varphi}$ (MTC): same, 6.vi.2000, leg. M. Toledo; $5 \ _{\varphi} \ _{\varphi}$ (MTC): same, 23.–28.iv.1998, leg. M. Toledo; 3.vi.2003, leg. M. Toledo; $2 \ _{\varphi} \ _{\varphi}$ (MTC): Mantova prov., Canneto sull'Oglio, Gerre Gavazzi, oxbow lake of river Oglio, 3.vi.2003, leg. M. Toledo; $2 \ _{\varphi} \ _{\varphi}$ (MTC): Mantova prov., Canneto sull'Oglio, Runate, oxbow lake of river Oglio, 28.iv.2003, leg. M. Toledo. EMILIA ROMAGNA: $1 \ _{\varphi} \ _{\varphi}$ (MTC): Parma prov., Parma, rain puddles on University Campus, 18.vi.2008, leg.

M. Toledo; 8 exs. (MTC): same, 22.iv.2009, leg. M. Toledo; 15 $_{\varphi,\varphi}$ (MTC, NHML): Parma prov., Salsomaggiore Terme, Stirone natural park, loc. Molinazzo, flooded maize field along rd. to Ponte Scipione, 140 m, 25.v.2008, leg. M. Toledo & R. Angus; 8 $_{\varphi,\varphi}$ (MTC): Parma prov., Varano de'Melegari, river Pessola on rd. to Castelcorniglio, 170 m, 21.v.2009, leg. M. Toledo; 3 $_{\varphi,\varphi}$ (SRC): Modena prov., Fanano, lago di Pratignano, 1307 m, 4.vi.2009.

TOSCANA: 10 $_{\varphi \ \varphi}$ (MTC): Pisa prov., Montecatini val di Cecina, small pond on road to Gello, near the cemetery, 200 m, 26.v.2009, leg. M. Toledo.

This species is common in N–C Italy and it occurs in various kinds of habitat, from sea level up to lower mountains. A chromosomal investigation on specimens collected from the Stirone natural park revealed triploid females (ANGUS & FOSTER 2009) while material from Parma's University Campus (see above) included diploid males (Angus, unpublished data), so that triploid and diploid conditions exist in Italian *brevipalpis* as proved for Spanish populations (ANGUS 1992b). Nevertheless it is not known whether diploid specimens coexist among triploid and parthenogenetic ones, or they make populations on their own. What is it suggested by the rest of the material examined, on the basis of the occurrence or not of males, is that north of the river Po (Lombardia) only parthenogenetic populations occur, being composed of females only (see material examined); in the plain south of the river Po and on the Northern Apennines the situation is more confused, with both parthenogenetic and non parthenogenetic populations, without a well defined pattern of distribution. The suspected parthenogenetic specimens collected also in Toscana (see above) suggest a similar situation also in central Italy, but much more material is needed to confirm this.

Helophorus (Rhopalhelophorus) strigifrons THOMSON, 1868

Material examined:

2 o o (FPC, NHML): VENETO: Verona, Pellegrina, Railway station, 12.iv.1964, leg. F. Pederzani.

This is a northern and central European species whose distribution extends into Siberia as far as the Irkutsk region, and which is not known from the Mediterranean or the Transcaucasus (ANGUS 1992a). This confirms the identification of CHIESA (1959) from Mantova and Pellegrina, which actually still represent the only known localities from Italy.

Helophorus (Rhopalhelophorus) lapponicus THOMSON, 1854

Material examined:

10 exs. (NMHL, FPC): UMBRIA: Monti Sibillini, Castelluccio di Norcia, Piano Grande and Piano Piccolo, 29-30.v.1964, leg. F. Pederzani; 8 exs. (MTC): same locality, Piano Grande, 30.v.2010, leg. M. Toledo.

This is a northern species whose main distribution extends from Scandinavia to eastern Siberia, but with glacial relict populations in the mountains of Spain, Turkey and the Transcaucasus, Israel/Lebanon and northern Iran (ANGUS 1992a). This is the first record of the species in Italy. It is not known from the Alps.

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Helophorus (Rhopalhelophorus) flavipes FABRICIUS, 1792, and *Helophorus (Rhopalhelophorus) obscurus* MULSANT, 1844

Material examined:

H. flavipes: 3 exs. (MTC): LOMBARDIA: Brescia prov., Gianico, Lago di Rondeneto, 1890 m, 5.vi.1983, leg. M. Toledo; 1 ex. (MTC): Brescia prov., M. Maniva, loc. Crocette, bogs, 2180 m, 2.viii.1982, leg. M. Toledo. TRENTINO-ALTO ADIGE: 4 exs. (MTC): Trento prov., Bresimo, Malga Bordolona di Sopra, 5.vi.1983, leg. A. Schizzerotto.
EMILIA-ROMAGNA: 4 exs. (MTC): Piacenza prov., Ferriere, Passo dello Zovallo, 1410 m, wetland made by tributary of stream Nure. 27 v 1998, leg. M. Toledo: 2 d d (NHML): Parma prov. Corniglio, riserva Guadine.

tributary of stream Nure, 27.v.1998, leg. M. Toledo; 2 & d (NHML): Parma prov. Corniglio, riserva Guadine Pradaccio, torbiera Roccabiasca, 1440 m, 24.v.2009, leg. G. Nardi & M. Bardiani.

H. obscurus: EMILIA-ROMAGNA: 1 ex. (MTC): Piacenza prov., Ferriere, Passo dello Zovallo, 1410 m, wetland made by tributary of stream Nure, 27.v.1998, leg. M. Toledo; 5 exs. (MTC, NHML): Parma prov., Salsomaggiore Terme, Stirone natural park, loc. Molinazzo, flooded maize field along rd. to Ponte Scipione, 140 m, 25.v.2008, leg. M. Toledo & R. Angus; 4 exs. (MTC): Parma prov., Berceto, M. Cervellino, spring, 1050 m, near Cascina La Sorte, 30.v.1997, leg. M. Toledo; 1 ex. (MTC): Parma prov., Viarolo, lents of Viarolo, 19.i.1992, leg. M. Toledo; 2 exs. (MTC): Modena prov., Fanano, lago di Pratignano, 1307 m, 4.vi.2009.

TOSCANA: 2 exs. (MTC): Pisa prov., Montecatini val di Cecina, "botro" [forest stream] on road Riparbella – Castellina Marittima, 9.v.2008, leg. M. Toledo; 3 exs. (MTC): Pisa prov., S. Luce, "botro" [forest stream] dei Castagni, rd. S. Luce – Castellina Marittima, 3.x.2007, leg. M. Toledo.

ABRUZZO: 2 exs. (MTC): Pescara prov., river Tavo near Farindola, 800–1100 m, 2.viii.1995, leg. M. Toledo; 4 exs. (MTC): Pescara prov., Serramonacesca, river Alento, 6 km S Serramonacesca, 26.v.2008, leg. M. Toledo; 1 ex. (MTC): Pescara prov., Manoppello, streamlet, ca. 270 m, road Manoppello – Serramonacesca, 26.v.2008, leg. M. Toledo; 5 exs. (MTC): L'Aquila prov., Scanno, streamlet, 1100 m, road Scanno – Villetta Barrea, 4 km S Scanno, 27.v.2008, leg. M. Toledo; 6 exs. (MTC): L'Aquila prov., Alfedena, La Montagnola, Rifugio Campitelli, 1566 m, wetland, 27.v.2008, leg. M. Toledo.

Confusion still occurs about the identity of these two species in Italy. The distribution given in ROCCHI (2005) for both *H. flavipes* and *H. obscurus* needs to be revised, being based mostly on old or historical records, when the two taxa where considered conspecific. Based on the data in our possession, *H. obscurus* appears to be much more common and widespread in Italy than *H. flavipes* and old records related to this latter species, in most cases should be attributed to *H. obscurus*, except, perhaps, for the Alps. According to ANGUS (1973) and HANSEN (1987) *H. flavipes* lives predominantly in acid waters, whereas *H. obscurus* is a neutral or basic water species. This agrees with a more localised presence of the former in Italy. The only Alpine records of *H. obscurus* formally known in Italy, are few localities on the western range (ROCCHI 2005); in our opinion these data are reliable due the closeness to the northern Apennines, where it is common. Elsewhere on the Alps this species might not occur.

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