RESEARCH ARTICLE

The identity of *Scambus planatus* (Hartig, 1838) and *Scambus ventricosus* (Tschek, 1871) as seasonal forms of *Scambus calobatus* (Gravenhorst, 1829) in Europe (Hymenoptera, Ichneumonidae, Pimplinae, Ephialtini)

Mark R. Shaw[1], Malcolm T. Jennings [2], Donald L. J. Quicke[3]

1 *Honorary Research Associate, National Museums of Scotland, Chambers Street, Edinburgh, EH1 1JF, U.K.*
2 *206 Lower Higham Road, Gravesend, Kent DA12 2NN, U.K.* **3** *Department of Biology, Imperial College London, Silwood Park Campus, Ascot, Berks SL5 7PY, UK, & Department of Entomology, Natural History Museum, Cromwell Road, London SW7 5BD, U.K.*

Corresponding author: *Mark R. Shaw* (markshaw@xenarcha.com)

Abstract

Through both natural and experimental rearings, supported by DNA sequence data (CO1, ITS2 and EF1α) as well as examination of material in collections, it is shown that the European nominal taxa *Scambus planatus* and *Scambus ventricosus* comprise the spring-emerging generation of *Scambus calobatus*, the typical form of which occurs as adults in late summer and autumn. The "*planatus*" form is shown to be the teneral (unhardened) version of the "*ventricosus*" form. The following synonymies are proposed: *Scambus calobatus* (Gravenhorst, 1829) = *S. planatus* (Hartig, 1838), **syn. n.** = *S. ventricosus* (Tschek, 1871), **syn. n.** Molecular data point to incidental natural rearings of a further two species, represented by single specimens.

Keywords

Parasitoid, synonymy, *Quercus*, acorns, *Cydia*, *Curculio*, *Acrobasis*, *Microtypus*, *Janus*

Introduction

Scambus calobatus (Gravenhorst, 1829) and *Scambus planatus* (Hartig, 1838) are two rather distinctive nominal taxa in the European fauna of around 30 species in the genus

Scambus Hartig. The two have been recognised as closely related (Perkins 1943) and share various apparent features (cf. Fitton et al. 1988), including rather light brownish metasomal tergites, that set them apart from most others; but they differ rather sharply in that *S. planatus* has substantially broader temples (compare Figs 1and 2), and also a shorter ovipositor than *S. calobatus* (ca 2.4–2.9 times as long as the hind tibia in *S. planatus*, as opposed to 2.9–3.7 in *S. calobatus*). In the following account the names *S. calobatus*, *S. planatus* and *S. ventricosus* (Tschek, 1871) are used in the sense of nominal taxa. Our interpretations follow authors who examined the relevant types: Perkins (1943) for *S. calobatus* and *S. planatus*, and Horstmann (2009) for *S. planatus* and *S. ventricosus*.

In the course of curating the large collection of Pimplinae in the National Museums of Scotland (NMS) it was noticed by the first author that British specimens determined as *S. planatus* all had spring dates (iii-v) of capture or emergence (especially from *Quercus* acorns collected the previous autumn, some harbouring larvae of the tortricid moth *Cydia*, probably *splendana* (Hübner), and others the curculionid beetle *Curculio*, probably *glandium* Marsham). In contrast, those determined as *S. calobatus* were either captured later in summer and autumn (vii-x) or reared around vii (especially from the phycitine pyralid moth *Acrobasis consociella* (Hübner), or from cocoons of its parasitoids, in spinnings among the foliage of *Quercus*). Despite the morphological differences, it seemed possible that the lack of information on a complete annual life cycle for either species might be explained by their being seasonal forms of a single entity–that is, the *calobatus* morph parasitizing hosts in acorns in autumn, from which adults of the *planatus* morph, with the powerful head musculature needed to chew out of acorns, emerge the following spring to attack hosts in leaf spinnings, from which the narrower-templed *calobatus* morph hatches in the same summer. When the apparent seasonality of the two forms in Britain was put to Klaus Horstmann he kindly examined material in some large German collections and informed us (in litt.) that the same was true there.

The view that only one species might be involved was reinforced by the collection of a morphometrically typical female *S. calobatus* walking among fallen acorns on 14.x.1999 (leg. M. T. Jennings, det. M. R. Shaw). Additionally, Bauer (2002) makes reference to seeing a female *S. calobatus* with its ovipositor in an acorn in the autumn (however, the specimen appears not to be in the Bauer collection: K. Horstmann, in litt.).

Because good numbers of both *S. planatus* (ex acorns) and *S. calobatus* (ex *Acrobasis*) could be obtained from woodland habitats in Kent (S. England), near the home of the second author, it was possible to embark upon testing the hypothesis that only one species was involved: firstly by rearing series of both nominal taxa from the same locations, and obtaining gene sequence data (organised by the third author); and secondly by rearing experiments, using spring-emerging *S. planatus* from acorns and experimental hosts such as *Acrobasis*, and then assessing the morphology of the resulting progeny. A chance capture of a free-flying spring female in a French locality provided an additional opportunity.

Part of the way through this process, the situation was made more complicated by Horstmann's (2009) raising of *Scambus ventricosus* (Tschek) from the synonymy of *S. planatus* (Hartig) that had been first proposed by Perkins (1943) and subsequently widely accepted. Horstmann (2009) cited as differences that *S. ventricosus* has a darker (mainly black) and less slender, more oval shaped metasoma with broader tergites than *S. planatus*, and also has a later flight period extending well into the summer (v-vii as against iii-v for *S. planatus*). According to Horstmann (2009) there are no host records for *S. ventricosus*, and the male is unknown.

Materials and methods

All rearings and other manipulations were done under approximately natural conditions of temperature and daylength (U.K.: Kent and Edinburgh).

In the period 1999–2009 much material of both *S. planatus* and *S. calobatus* was reared by the second author from a series of more or less proximate woods in Kent, England (OS map refs [site names]: TQ6666 [Luddesdown]; TQ6667 [Cobham]; TQ6865 [Halling Wood]; TQ6870 [Shorne]; TQ7174 [Lower Higham] and TQ7876 [Northward Hill]) and sent to the first author for determination. *Scambus planatus* emerged as a primary parasitoid of both *Cydia* and *Curculio* in spring (ca iv) from fallen acorns collected the previous autumn (ca x-xi) that had been kept over winter in an unheated shaded outhouse (cf. Shaw 1997). *Scambus calobatus* emerged from webs of *Acrobasis consociella* fairly soon after they were collected in about vi-vii, both as a primary parasitoid and as a pseudohyperparasitoid from cocoons of the braconid primary parasitoid *Microtypus wesmaelii* Ratzeburg. Adults were killed into ethanol (for removal of a leg for gene sequencing) or allowed to die of starvation; in both cases the specimens were then mounted for morphological examination.

For experimental rearings, four females of *S. planatus* that emerged in spring 2010 from acorns collected the previous autumn in the above woods were isolated and thereafter kept separate (allowing all progeny to be related back to its mother). Males that emerged from the same collections were introduced to the females and left with them for up to four days. Observation was not continuous, but two of the females were seen to mate. The adult females were then kept in corked 7.5 × 2.5 cm glass tubes and fed ad libitum on honey:water. They were also offered a range of wild-collected microlepidopteran larvae (mainly Tortricidae) that had been removed from their leaf rolls and folds on *Quercus* and *Crataegus*. Some of the larvae were purposely injured with a pin prior to being offered to the females, to encourage host-feeding. Some behavioural observations were made, but observation was sporadic. Approximately 20–25 days after their emergence, the females were each transferred to a closed clear plastic box (15 × 11 × 7 cm) and offered about 6–8 wild-collected *Quercus* spinnings containing larvae (1–4 per spinning) of *Acrobasis consociella*. In all cases the spinning was first partly opened to verify that all the caterpillars that were present were fully active (this ensured that no hosts already parasitized by *Scambus* were inadvertently

introduced: *Scambus* species permanently paralyse the host prior to oviposition). The spinnings, which were quickly re-closed by the intended hosts, were left with the females for variable lengths of time, some being removed after one day and others after 2–3 days. Some that still contained active larvae were returned to the same female subsequently. The host larvae were left in situ and the accumulated spinnings that had been offered to each female were retained separately in the plastic boxes, now opened and covered with fine netting, and kept in an unheated shaded outhouse. The spinnings were occasionally lightly sprayed with water. Parasitoid adults that resulted from these experimental exposures were killed into ethanol, as were the females at the end of the runs (by which time they were ca 30 and in one case over 40 days old), and, after removal of a leg for gene sequencing, mounted. These events are referred to below as the "Kent experiments".

An additional, earlier, experiment involved a female caught on 28.v.2008 flying around *Quercus* in France near Chinon (Indre-et-Loire), that was from that date fed ad libitum on honey:water and continuously offered larvae of the tortricid *Acleris quercinana* (Zeller) in situ in their spinnings as potential hosts. Both the parent and her offspring were subsequently dealt with as above. This is referred to as the "Chinon experiment".

Specimens from field and from experimental rearings were sequenced for the 5' bar-coding region of the mitochondrial cytochrome oxidase 1 gene following the protocols in Kuhlmann et al. (2007) or performed by the BarCodingLife project. Some field-reared specimens were additionally sequenced for the nuclear internal transcribed spacer 2 gene (ITS2) region and the nuclear elongation factor 1α 5' intron (EF1α), also following Kuhlmann et al. (2007). Sequences are available from Genbank (accession numbers JN243100-JN243134).

All *Scambus* specimens resulting from this study are deposited in the National Museums of Scotland, Edinburgh.

Results

In the Chinon experiment, the female behaved as a predator of several *Acleris quercinana* larvae, probing their leaf rolls vigorously with her ovipositor, mutilating the larvae and imbibing their fluid content, but she did successfully parasitize one (on or around 1.vi.2008) and an adult female *Scambus* resulted early in vii.2008 (during a period when it was not possible to check the box). The parent (which must have been mated) and her daughter had identical CO1 sequences (Genbank accession numbers JN243114 and JN243115; see Table 1), and the offspring was very clearly morphologically a normal *S. calobatus* (Fig. 1) while in head and ovipositor structure the parent was indistinguishable from *S. planatus* (Fig. 2). The specimens were shown to Klaus Horstmann, largely as a courtesy because of the interest and help he had given, but he determined the parent female as *Scambus ventricosus*, not *S. planatus*. This raised the possibility that *S. planatus* and *S. ventricosus*, at the time believed

Table 1. Specimen codes, parentage relations and GenBank DNA sequence accession details.

Specimen voucher code	Collection locality	Morpho-type	Sex and notes on parentage	Genbank CO1 accession number	Genbank ITS2 accession number	Genbank EF1α accession number
Scambus20104 BCLDQ01664	UK, Kent, Northward Hill	*planatus* becoming *ventricosus*	female, parent of Scambus20105	JN243100	-	-
Scambus20105 BCLDQ01665	UK, Kent, Northward Hill	*calobatus*	male, son of Scambus20104	JN243116	-	-
Scambus20106 BCLDQ01666	UK, Kent, Halling Wood	*planatus* becoming *ventricosus*	female, parent of Scambus20107	JN243102	-	-
Scambus20107 BCLDQ01667	UK, Kent, Halling Wood	*calobatus*	male, son of Scambus20106	JN243101	-	-
Scambus20101 BCLDQ01661	UK, Kent, Northward Hill	*planatus* becoming *ventricosus*	female, parent of Scambus20103	JN243104	-	-
Scambus20103 BCLDQ01663	UK, Kent, Northward Hill	*calobatus*	male, son of Scambus20101	JN243103	-	-
MJ1950-I	UK, Kent, Northward Hill	*calobatus*	male, ex *Microtypus wesmaelii* cocoon in *Acrobasis consociella* retreat	JN243107	JN243119	JN243128
MJ1973E-G	UK, Kent, Northward Hill	*calobatus*	female, ex *Acrobasis consociella*	JN243108	JN243120	JN243129
MJ2047c-F	UK, Kent, Northward Hill	*planatus*	male, reared from fallen acorn	JN243109	JN243121	JN243130
MJ2047b-E	UK, Kent, Northward Hill	*planatus*	female, reared from fallen acorn	JN243110	JN243122	JN243131
MJ2047a-D	UK, Kent, Northward Hill	*planatus*	female, reared from fallen acorn	JN243111	JN243123	JN243132
MJ2041-C	UK, Kent, Cobham	*planatus*	female, reared from fallen acorn	JN243112	JN243124	JN243133
MJ2038-B	UK, Kent, Halling Wood	*planatus*	female, reared from fallen acorn	JN243113	JN243125	JN243134
BCLDQ0370	France, Chinon	*ventricosus*	wild caught female, parent of BCLDQ0371	JN243114	-	-
BCLDQ0371	France, Chinon	*calobatus*	female, daughter of BCLDQ0370	JN243115	-	-
MJ2073-A	UK, Kent, Lower Higham	[near to but not *planatus*]	female, ex J*anus femoratus* in oak twig gall	JN243105	JN243117	JN243126
MJ1970-K	UK, Kent, Northward Hill	cf. *inanis*	male, ex *Acrobasis consociella*	JN243106	JN243118	JN243127

to be species distinct from one another, both had (indistinguishable) late summer "*calobatus*" morphs. This necessitated the Kent experiments, using *S. planatus* female parents of known origin.

Figures 1, 2. Head, dosal aspect, of *S. calobatus* morphs (female). 1, "*calobatus*". 2, "*planatus/ventricosus*" (equivalent in this respect). The specimen in Fig. 1 is the offspring of that in Fig. 2, from the "Chinon experiment" (see text); respectively BCLDQ0371 and BCLDQ0370 in Table 1.

In the Kent experiments, all four females host-fed avidly and destructively on a succession of tortricid and possibly other microlepidopteran larvae for ca 20 days. The females were quickly attracted to the introduced larva and attacked it with their ovipositor or, more commonly, directly with their mandibles. When larvae that seemed too vigorous or large to be overcome were removed, injured with a pin, and returned, the female *Scambus* would also often imbibe the host's fluid content completely. When the females were eventually offered *A. consociella* spinnings they actively searched for hosts and paralysed and oviposited onto *A. consociella* larvae freely. One spinning, when partly opened, revealed a paralysed larva with at least five white elongate *Scambus* eggs either attached or immediately adjacent to the host. However, it is likely that this was an unusual degree of superparasitism resulting from the limited availability of host larvae and excessive exposure time. Destructive host-feeding was also observed on some of the *A. consociella* larvae offered in situ. Parasitoid adults emerged 25–32 days after the females were first offered *A. consociella* hosts. The progeny obtained from the four females was respectively: 3 ♀, 9 ♂; 1 ♀, 8 ♂; 7 ♂; and 4 ♂. CO1 sequences for parent female and (one) male progeny were identical in all three cases for which sequences were obtained from both mother and son (Genbank accession numbers JN243100-JN243104 and JN243116; see Table 1) and all progeny of both sexes conformed in all morphological respects to *S. calobatus*.

DNA sequence data (CO1, ITS2 and EF1α) were also obtained from wild-reared specimens from the Kent sites, comprising three individuals reared from *Acrobasis consociella*, five reared from fallen acorns and one reared from a gall of the cephid sawfly *Janus femoratus* (Curtis) in an oak twig (Table 1). The specimen (MJ2073-A) reared from the sawfly host and one small (runtish) male reared from *Acrobasis* (MJ1970-K) will be discussed briefly below. The other specimens, including those involved in the experimental rearings and whether representing *calobatus* or *planatus* morphologies, were all identical with respect to their CO1 genes and, for those individuals for which it was sequenced, their ITS2 genes were similarly identical. For EF1α, there were 11

positions where single bases differed between individuals with no clear patterns in relation to their morphology. In addition, at the length-variable T-rich insertion the number of Ts in the run varied from eight to 12 between individuals, also showing no correlation with morphotype.

The two above-mentioned specimens MJ2073-A and MJ1970-K differed markedly from the *calobatus/planatus* above. In the CO1 gene fragment both differed in the same way from the *calobatus/planatus* ones at seven bases, differed in different ways at one further position, and individually differed from *calobatus/planatus* at another five and six base positions respectively. For ITS2, MJ1970-K and MJ2073-A differed from *calobatus/planatus* at six and one base respectively. For the alignable parts of the EF1α region (420 out of 425 base pairs), MJ1970-K differed from the *calobatus/planatus* sequences at 10 positions, MJ2073-A at one position and both at a further two positions. At the T-rich insertion MJ1970-K had only six Ts whereas MJ2073-A had a run of 18 Ts and, in addition, MJ1970-K had a unique 10 base-pair insert towards the 3' end.

Discussion

The Kent experiments clearly showed that the nominal species *S. planatus* is just the spring-emerging morph of *S. calobatus*, but the earlier Chinon experiment that had also resulted in *S. calobatus* progeny, but from a *S. ventricosus* parent, remained at first sight puzzling. However, examination of the female parents from the Kent experiments, killed after their lengthy period of feeding to mature their eggs (and subsequent ovipositions), immediately resolved the paradox, because these parents now had fully black tergites that were also flatter (i.e. broader) (Figs 3, 4) and resulted in a more oval metasoma than the other *S. planatus* adults, which had similarly originated from acorns at the same sites but had been killed unfed soon after emerging (Figs 5, 6). The four aged females had thus become indistinguishable from *S. ventricosus*, and it was clear that the remaining supposed differences between this nominal species and *S. planatus* were all perfectly explained by this observation (i.e. the later flight time of *S. ventricosus*; for which there is no known host as all reared specimens would be in the *planatus* morph on emergence; nor known males as they are presumably too short-lived to attain the *ventricosus* state).

Obtaining the molecular data was embarked upon before the rearing experiments could be set up, but the molecular results are given for the sake of completeness; in the light of the experimental data they serve largely to remove any conceivable doubt that contaminants, in the form of already parasitized hosts, had been introduced.

In fact, it might have been deduced that the females of the *S. planatus* morph (and indeed *S. calobatus*) are teneral on emergence and far from able to parasitize hosts, from the state of their ovipositors. Freshly emerged individuals invariably have the ovipositor shaft almost white (only the tip darkened to brownish) (Fig. 7), and soft enough for it to curl and exfoliate into its component valves very easily. Moreover, their rather pale tergites are substantially rolled and not rigid. Although

Figures 3–6. Metasoma (3, 5) and 2nd metasomal tergite (4, 6), dorsal aspect, of *S. calobatus* morphs (female). **3, 4** "*ventricosus*" (one of the aged female parents after use in the "Kent experiments" (see text)). **5, 6** "*planatus*" (a specimen starved to death from emergence). In both cases reared from acorns at the Kent sites.

most ichneumonoids rest as adults for a period in their cocoon to emerge in a more or less fully mature state, this trait of teneral emergence is not uncommon in ephialtine Pimplinae (possibly associated with the lack of a strong cocoon, but in any case particularly easily detected in that group because of the often long ovipositors of the females), and the changing morphology with maturity that is seen in *S. calobatus* and its *planatus* morph should be noted as a hazard for taxonomists that may have a wider currency–as, indeed, has been discussed very clearly in relation to Ephialtini by Perkins (1943). Re-examination of the female *S. calobatus* found walking among fallen acorns on 14.x.1999 (see Introduction) revealed that it had a well-darkened ovipositor shaft and also mostly black tergites (deep chestnut at the sides), quite different from the individuals killed soon after being reared from *Acrobasis consociella*.

Conclusion

In the light of these findings we formally propose the following new synonymy of nominal taxa currently placed in *Scambus*: *Pimpla calobata* Gravenhorst, 1829 (senior name) = *Pimpla (Scambus) planata* Hartig, 1838, syn. n. = *Pimpla ventricosa* Tschek,
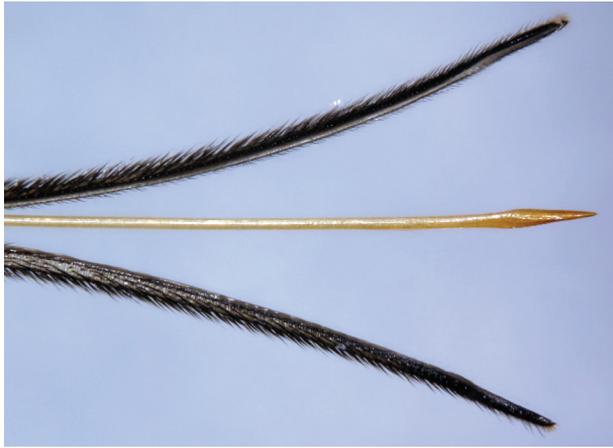
Figure 7. Ovipositor, dorsal aspect, of *S. calobatus* ("*planatus*" morph) killed within 2 days of egress from an acorn.

1871, syn. n. The informal name "form *planatus*" might still be useful as a label for the spring generation morphotype of *S. calobatus*.

There is no modern revision of *Scambus* and, because of the large divergence in ovipositor length, temple width, tergite width and even colour of the morphs of *S. calobatus*, it is not easy to give characters that will define the species as a whole. Thus, at least for now, it will be necessary to continue to determine the seasonal morphs separately, using existing keys. However, in the male sex the rather broad genital claspers that are partly covered with fine longitudinal striae is a consistent character that may not occur in other European species (K. Horstmann, pers. comm.).

Regarding the anomalous specimens, K. Horstmann (pers. comm.) confirms that, morphologically, the female specimen MJ2073-A differs sufficiently from the *planatus* morphotype (to which it comes closest) that he would regard it as a different, probably undescribed, species. The runtish male specimen MJ1970-K, with an even greater level of molecular difference, almost certainly represents a further species, perhaps *S. inanis* (Schrank) which is commonly reared in Britain from hosts in leaf rolls and leaf mines on deciduous trees (Shaw 2006), but whose males are sometimes morphologically hard to determine unequivocally (Horstmann 2005).

Acknowledgements

References

Bauer R (2002) Bemerkungen über die Ichneumoniden der Alpen Teil IV (Hymenoptera, Ichneumonidae). Entomofauna 23: 93–107.

Fitton MG, Shaw MR, Gauld ID (1988) Pimpline ichneumon-flies. Hymenoptera, Ichneumonidae (Pimplinae). Handbooks for the Identification of British Insects 7(1): 1–110.

Gravenhorst JLC (1829) Ichneumonologia Europaea Pars III. Vratislaviae: 1–1097.

Hartig T (1838) Ueber den Raupenfrass im Königl. Charlottenburger Forste unfern Berlin, während des Sommers 1837. Jahresberichte über die Fortschritte der Forstwissenschaften und forstlichen Naturkunde nebst Originalarbeiten aus dem Gebiete dieser Wissenschaften, Berlin 1 (1836 und 1837) [1837–1839], Heft 2: 246–274.

Horstmann K (2005) Über einige mit *Scambus inanis* (Schrank, 1802) nah verwandte Arten (Hymenoptera, Ichneumonidae, Pimplinae). Entomofauna 26: 101–115.

Horstmann K (2009) Revisionen von Schlupfwespen-Arten XIII (Hymenoptera: Ichneumonidae). Mitteilungen der Münchener Entomologischen Gesellschaft 99: 37–44.

Kuhlmann M, Else GR, Dawson A, Quicke DLJ (2007) Molecular, biogeographical and phenological evidence for the existence of three western European sibling species in the *Colletes succinctus* group (Hymenoptera: Apidae). Organisms Diversity & Evolution 7: 155–165. doi: 10.1016/j.ode.2006.04.001

Perkins JF (1943) Preliminary notes on the synonymy of the European species of the *Ephialtes* complex (Hym.), Ichneumonidae. Annals and Magazine of Natural History (11) 10: 249–273.

Shaw MR (1997) Rearing Parasitic Hymenoptera. Amateur Entomologist's Society (Orpington): 1–46.

Shaw MR (2006) Notes on British Pimplinae and Poemeniinae (Hymenoptera: Ichneumonidae), with additions to the British list. British Journal of Entomology and Natural History 19: 217–238.

Tschek C (1871) Ichneumonologische Fragmente. Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien 21: 37–68.