Description of *Aprostocetus anoplophorae* n. sp. (Hymenoptera: Eulophidae), a new egg parasitoid of the invasive pest *Anoplophora chinensis* (Förster) (Coleoptera : Cerambycidae)

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Abstract – *Aprostocetus anoplophorae* n. sp. (Hymenoptera: Eulophidae) is supposed to play a role as an egg parasitoid of the invasive pest, the Citrus Longhorned Beetle, *Anoplophora chinensis* (Förster). The studies of its morphology, and rDNA sequence data, strongly indicate that this taxon differs greatly from all described *Aprostocetus* species, and is new to science. This species is described and illustrated. Both its systematic placement and origin are discussed.

Résumé – **Description de *Aprostocetus anoplophorae* n. sp. (Hymenoptera, Eulophidae), un nouveau parasite de l'espèce invasive *Anoplophora chinensis* (Förster) (Coleoptera, Cerambycidae).** – *Aprostocetus anoplophorae* n. sp. (Hymenoptera : Eulophidae) est supposée jouer un rôle comme parasite des œufs de l'espèce de Cerambycidae invasive *Anoplophora chinensis* (Förster). L'étude morphologique et les séquences ADNr indiquent fortement que ce taxon est très different des autres espèces décrites d'*Aprostocetus* et est nouvelle pour la science. Elle est décrite et illustrée. Sa position systématique et son origine sont discutées.

Two longhorned beetles *Anoplophora glabripennis* (Motschulsky), and *Anoplophora chinensis* (Förster) (= *malasiaca*) (Coleoptera, Cerambycidae) have been accidentally introduced in North America (Haack *et al*. 1997; Cavey *et al*. 1998) and Europe (Dauber & Mitter 2001; Colombo & Limonta 2002; Cocquempot & Hérard 2003; Cocquempot *et al*. 2003a; 2003b). In 2000, the presence of *A. chinensis* was reported at Parabiago (MI), Italy, in the neighborhood of a nursery where bonsais imported from Eastern Asia were grown. In 2003, *A. chinensis* was detected at Soyons, France, and hence was considered as an invasive pest, subject to eradication. Both species are originated from Eastern Asia where they cause serious damages to many deciduous trees, mainly in the genera *Populus*, *Acer* and *Salix*. They also attack *Aesculus hippocastanum*, and species of *Betulus*, *Fraxinus*, *Morus*, *Pyrus* and *Robinia*. *A. chinensis* is a major pest of *Citrus* spp. trees in Japan (Adachi 1994). Both pests are considered as serious threats to the urban and natural forests in North America and Europe. Therefore, the U.S. government started an eradication program by destroying all the infested trees in New York, Long Island, Jersey City, and Chicago

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areas. In conjunction with the eradication program, biological control studies were initiated in order to find, to identify, and evaluate the parasitoids that could successfully control *Anoplophora glabripennis* and *A. chinensis* (Hérard *et al*. 2002, 2004; Smith *et al*. 2002, 2004). Recently a survey was attempted to find possible new associations between introduced *Anoplophora* and natural enemies of some European cerambycids that share similarities with these Asian pests in terms of taxonomy, host-plant choices, and behaviors. *Saperda* spp. are among these species. In this context, explorations for eggs and early stage larvae parasitoids were made in various European countries where *Saperda* spp. (Col., Cerambycidae) occurred and where the two Asian pests were first detected.

In February 2002, at Parabiago, for the first time, two eggs of CLB were found which had been attacked by a gregarious parasitoid. A thorough review of the potential taxa which could share the same host and ecology as this parasitoid was made. Among the different families that could fit in, Eulophidae which represents a large and biologically varied family of parasitoid wasps, was proposed. Given the small chance for the larvae to achieve their complete development, the hibernating full grown parasitoid larva from one of the two host eggs was genetically characterized using DNA-based markers, the larvae from the other egg being reared to adulthood. It was thought that molecular biological techniques might help in the taxonomic identification of this parasitoid. Recent phylogenetic studies using comparative morphology and DNA sequence data have greatly enhanced the possibility of species diagnostic assays (Hillis *et al*. 1996; Heraty 2003). Only in recent years, has the phylogeny of Eulophidae been studied using sequence data of the second expansion segment (D2) of the 28S ribosomal subunit (Gauthier *et al*. 2000). At first assessment, such phylogenetic classification provides a series of valuable data for both the taxonomic identification and position of unknown taxon.

This paper describes how the combination of both classical taxonomy and the comparison between both the sequence of the target region D2-28SrDNA for this larval specimen and those from representatives of the Eulophidae, have provided important clues regarding the taxonomic status of this parasitoid. At this stage of the study, assessing the identity of the parasitoid was of primary importance for determining whether the parasitoid had been introduced or even established in Italy, together with its exotic host; or, was the result of a new association with the Asian host. Detailed morphological comparisons with Asian *Aprostocetus* spp. led to the conclusion that this egg parasitoid of *Anoplophora chinensis* belongs to an undescribed species.

Material and methods

Morphology – Adult specimens of *Aprostocetus anoplophorae* described here were reared from *Anoplophora chinensis* eggs collected at Parabiago, 32 km North-West of Milan, Lombardy; Italy (45° 34' N; 8° 57' E). The specimens were killed several hours after emergence, kept in ethanol 70° for some weeks, then critical point dried (CPD) and mounted on cards. Measurements were made with a Wild M5 stereomicroscope. These correspond to the maximal dimensions of sclerites or appendages; lengths for the mesosoma and gaster were measured in lateral view. Terminology follows that used by Graham (1987) except that thorax was replaced here by mesosoma. The measurements were made on such CPD specimens. The relative measurements mentioned are from the female holotype. Ratios were calculated from 5 females and 5 males.

Specimen collection and identification for DNA analysis – A living parasitoid larva, separated in February 2002 from a parasitized egg of *Anoplophora chinensis*, was preserved in 96° ethanol, and frozen at – 20 °C. Adult specimens of *Aprostocetus luteus* (Ratzeburg), *A. collega* (Ratzeburg), and *A. lycidas* (Walker) were reared from galls of the cecidomyiid *Hartigiola annulipes* (Hartig) on beech tree leaves (*Fagus sylvatica* L.) collected at the Plateau de L'Escandorgue, Lauroux, Hérault, France (43° 48' N; 3° 15' E); some adults were collected directly while they were visiting the galls. Collections were made in early October 2002. Freshly field captured, or emerged, specimens were transferred to 96° ethanol, identified by G. Delvare (CIRAD), and stored at – 20 °C. Adult specimens of *A. elongatus* (Förster) were collected on the eastern slope side of Mount Köszeg, near Köszeg city, Hungary, on May 2001.

DNA preparation, PCR and sequencing – Total genomic DNA was isolated as follows: The specimen was hydrated by immersion in sterile distilled water several times and crushed with a plastic pestle in 1.5 ml microcentrifuge tube containing 500 µl lysis buffer (2% cationic hexadecyl trimethyl ammonium bromide (CTAB), 0.7% M NaCl, 20 mM ethylenediaminetetra-acetic acid (EDTA), 1% PVP 360 w/v, 0.2 mg/ml proteinase K, 0.2% ß-mercaptoethanol, 100 mM Tris-HCl, pH 8.0. After 1 hour of incubation at 60°C, an equal volume of 25: 24: 1 phenol/chloroform/isoamyl alcohol was added, the tubes shaken thoroughly and then spun for 10 min. The aqueous layer was transferred to a new tube and the previous step repeated using an equal volume of 24: 1 chloroform/isoamyl alcohol. DNA was precipitated using 1/10 volume of 3 M NH4Ac and 2.5 volumes 100% ethanol, washed with 70% ethanol and resuspended in 20 µl of TE (10 mM Tris HCl, pH 8.0, 1 mM EDTA). The 28S-D2 region was amplified using the primers as described by Campbell *et al*., (1993): D2-forward primer 5'-AGTCGT-GTTGCTTGATAGTGCAG-3' and D2-reverse primer, 5'-TTGGTCCGTGTTTCAAGACGGG-3'.

Standard 25 µl PCR reactions were performed using 2U Taq Polymerase (Qiagen, S.A. France), 2.5 µl Qiagen PCR buffer (1.5 mM MgCl2), 0.4 mM dNTPs, 0.5 µM of each primer. Two µl of DNA was used as the polymerase chain reaction (PCR) template. Cycle conditions on a Hybaid PCR Express thermocycler were: initial denaturation of 3 min at 97°C; then 35 cycles

of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C; and a final extension of 7 min at 72 °C. Both strands of the purified PCR product were sequenced by Genome express (Meylan, France) on an ABI 3730 XL ™ automated sequencer. A consensus sequence was obtained after alignment of sequences of both strands using Sequence Navigator Editor. BLAST alignment searches using the BLASTN algorithm (BLASTN 2.0.13, NCBI Blast 2000, Altschul *et al*. 1990) were done to ascertain the identity of the sequences of the following *Aprostocetus* species (*A. luteus*, *A. collega*, *A. lycidas*, *A. elongatus*) and of the larvae reared from the egg of *Anoplophora chinensis* using closely related sequences already deposited in GenBank. Sequence alignment was performed using Clustal X program version 1.81 (Thompson *et al*. 1997). Distances between the *Aprostocetus* sequences were calculated based on the Hasegawa parameter (HKY85) using PAUP 4.0 b10 (Swofford 1998).

Abbreviations

POL Distance between posterior ocelli
OOL Distance between one posterior ocellus and the relevant compound eye

Taxonomy

Aprostocetus anoplophorae Delvare, sp. n.
(Figs. 1-10)

Female

2.5-3 mm. Body black. Head yellowish on the margin of the oral fossa, along the subtorular lines and the frontal lines. Tegula yellowish. Scape and pedicel yellowish but largely infuscate dorsally. Flagellum darkened. Leg yellowish with the apical half of the hind coxa and sometimes the hind femur dorso-medially infuscate. Wings hyaline with pale yellow veins. Ovipositor dark.

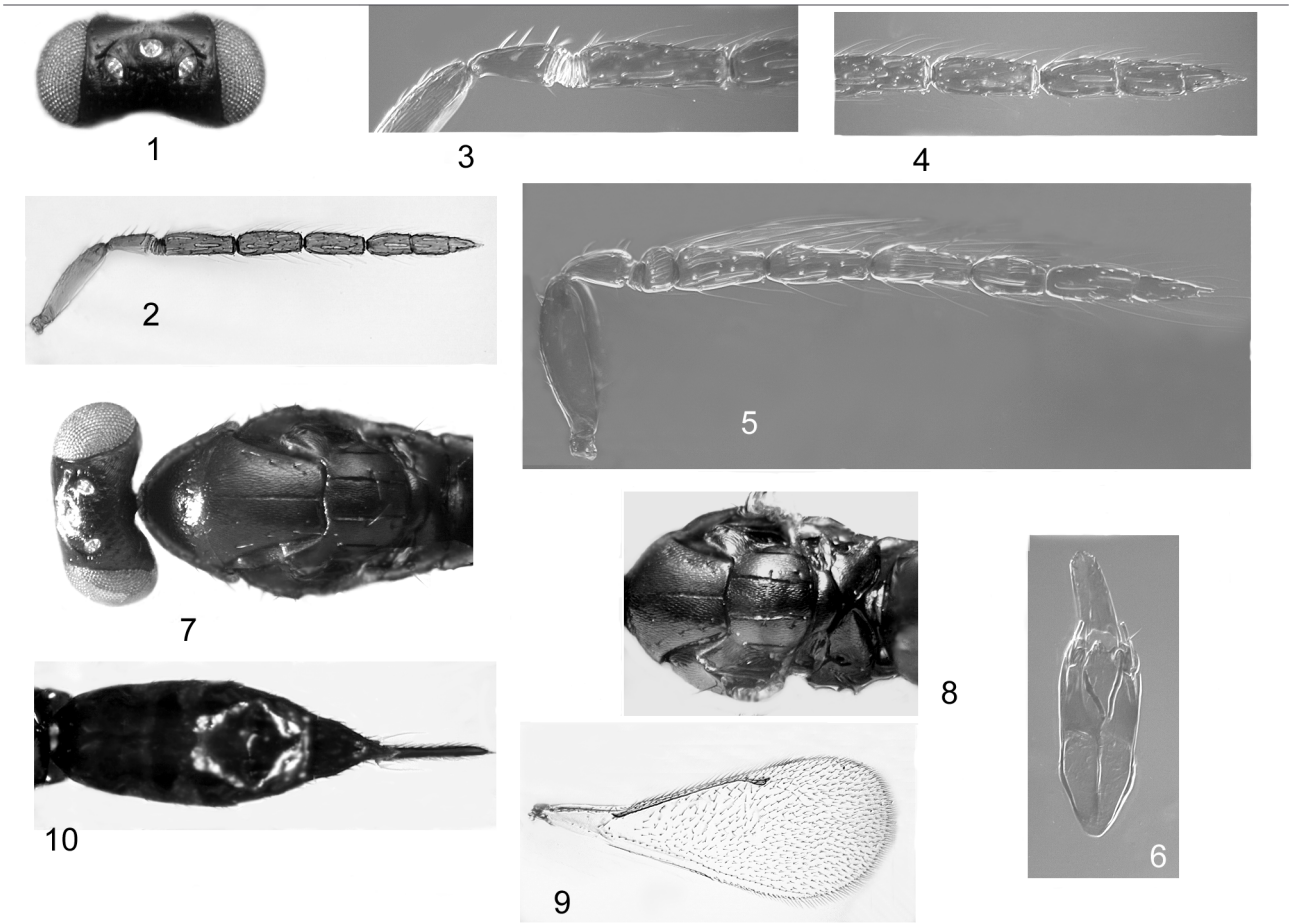
Head (Fig. 1-4). – Relative measurements. Head width 39, length 17.5 and height 30. Malar space 9, eyes 20: 16, distance antennal toruli-ventral margin of the clypeus 11, fronto-vertex 20, POL 8, OOL 3.7, lateral ocelli diameter 4, distance median-lateral ocelli 3. Relative measurements of antenna: Scape 15:4.5, pedicel + flagellum 59, pedicel 7:3.5, F1 11:4, F2 11:3.5, F3 10:3, clava 19. Head slightly broader than the mesoscutum (× 1.0-1.1), 1.23-1.32 times as broad as high and more than twice as broad as long (× 2.15-2.3). Oral fossa 1.6-1.62 length of malar sulcus, latter 0.41-0.45 eyes height. Eyes 1.2-1.3 times as high as long. POL 2.15-2.30 OOL. Lateral ocelli diameter slightly greater than the latter. POL 2.5-2.65 the distance between the median and lateral ocelli. Mandibules tridentate, the upper sometimes subtruncate. Malar sulcus moderately curved. Subtorular grooves present, subparallel. Ventral margins of the antennal toruli distinctly above the lower eye margin. Lower face and genae with excessively fine and superficial impressed network and bearing short hairs. Frontal lines as usual, T-like. Frons smooth, bearing longer and suberect hairs. Eyes apparently bare. Vertex and occiput with small piliferous punctures and appressed hairs. Scape 2.8-3.75 as long as wide, shorter than the height of the eyes (15: 20) and reaching very slightly above the vertex, bearing 3 hairs on its anterior margin. Pedicel plus flagellum much longer than the width of the mesoscutum (× 1.45-1.7). Pedicel 2-2.3 times as long as wide (in dorsal view) (Fig. 3). Flagellum long and thin, almost linear, not or hardly stouter than the pedicel, latter followed by 4 visible anelli (Fig. 2). F1 much longer than latter (× 1.5-1.65), 3-3.3 times as long as wide, F2 and F3 subequal 2.8-3.15 times as long as wide. Funicular segments with 2-4 imbricate rows of sensilla. Clava long and acuminate, 5.5-6.3 times as long as wide, and slightly shorter than F2 + F3 combined (× 0.82-0.87) (Fig. 4). No constrictions between its segments. Apical spine half as long as last segment, terminal seta as long as the spine.

Mesosoma (Fig. 7-9). – Length 56, width 35. Pronotum 15 (in lateral view), mesoscutum length 22, scutellum 17: 19, distance between its submedian lines 7 at base and 6 at apex, distance submedian-sublateral lines 5, dorsellum 4, propodeum on the median line 4. Forewing 110:47, fringe 3, costal cell 25, marginal vein 36, stigmal vein 9. Hind wing 90:17, fringe 5. Apical width of the mid tibia 3.5, length of the apical spur 5, basitarsus 12. Hind femur 31:8, hind tibia 39, apical spur 5, basitarsus 15, tarsus 34. Mesosoma relatively elongate, 1.45-1.67 times as long as broad. Pronotum long, 0.6-0.8 the mesoscutum (in lateral view), subconical in dorsal view, sloping at about 60° and bearing a subapical row of 6-8 erect setae (length = 4). Mid lobe of the mesoscutum with 3-5 pairs of adnotaular setae (first pair length 4.5, last pair length 6), median line complete. Impressed network very fine and superficial, forming elongate cells on its antero-median surface, progressively turning to isoedric to posterolateral surface. Mesoscutum longer than the scutellum (× 1.2-1.5) latter 0.70-0.89 as long as wide, distinctly convex. Impressed network similar to the mesoscutum. Relative position of the submedian lines quite variable, sometimes more distant from each other than from the sublateral lines, other times closer to each other; sometimes subparallel, other times slightly converging backwards. Anterior pair of setae at approximatively half length of the scutellum. Dorsellum smooth and slightly convex. Propodeum about as long as the dorsellum on the median line, sloping at about 45°, median carina somewhat broadened and flattened, surface of the propodeum almost smooth, the network hardly visible and delimiting isoedric cells. Callus bearing 2 setae (Fig. 8).

Forewing 2.25-2.35 times as long as broad, fringe 0.25-0.35 the stigmal vein (Fig. 9). Costal cell very narrow, bearing a row of 5-8 ventral hairs. Submarginal vein bearing 6-8 dorsal setae. Marginal vein 3.8-4.5 the stigmal, bearing 12-16 setae on its frontal edge. Stigmal vein at 40-45° from the margin of the wing, thin, with a long uncus. Basal vein with 2 hairs, speculum as long as the 4th or 5th of the marginal vein, closed behind with a line of 4-6 setae on the cubital fold. Hind wing 4.9-5.5 times as long as wide, narrowly rounded at apex, fringe 0.25-0.35 width of the wing.

Legs elongate and slender. Apical spur of the mid tibia short (0.4-0.6 length of basitarsus), but longer than the apical width of the tibia (× 1.4-1.8). Hind femur 3.7-3.9 times as long as broad.

Gaster (fig. 10). Length 91, width 30, epipygium 9, postcercale + ovipositor 32, tip of hypopygium 55 from base of the gaster. Gaster acuminate, 3-3.3 times as long as broad. Epipygium as long as wide, shorter than longest cercal seta which is regularly curved, not kinked. Ovipositor distinctly exserted with



Figures 1-10.
Aprostocetus anoplophorae n. sp. – 1, female head (dorsal view). – 2, female antenna. – 3, base of female antenna. – 4, apex of female antenna. – 5, male antenna. – 6, male *genitalia*. – 7, head and mesosoma of female (dorsal view). – 8, mesosoma of female (apex). – 9, female fore wing. – 10, female gaster.

length of postcercale + ovipositor 0.8-0.9 length of hind tibia. Tip of hypopygium at 0.6-0.7 length of gaster.

Male

Differs from the female in following respects: entire antenna yellowish, only scape broadly infuscate dorsally. Lateral ocelli diameter smaller than OOL. Scape somewhat flattened, only 2.65 times as long as wide, with ventral plate about two thirds length of scape (Fig. 5). Pedicel + flagellum about 1.5 width of mesoscutum. Pedicel 1.6 as long as wide. F1 shorter than pedicel. F2 3.7-3.8, F3 3.5 and F4 3.5 times as long as wide. Clava somewhat longer than F3 + F4 combined. Whorled setae of flagellomeres very long, those on F1 reaching mid length of F4. Terminal seta of clava very long and curved, as long as the third segment. Fore wing somewhat shorter, only 2.25 times as long as wide. Fringe as long as stigmal vein. *Genitalia* as in Fig. 6.

Material examined – ♀ Holotype: Italy (Lombardy, Milan): Parabiago, ex egg of *Anoplophora chinensis* (Col., Cerambycidae) under bark of *Acer saccharinum;* host egg collected on 18.III.2003; emergence of adult parasitoid on 27.VI. 2003 (Hérard Franck). EBCL ref. AC-PAR3-02; CIRAD ref. 18069 (deposited in Centre Inter-Organisme de Recherche et d'Expertise en Systématique, Montferrier-sur-Lez, Hérault, France). Paratypes. 17 ♀♀ and 6 ♂♂ with the same references. 7 ♀♀ and 1 ♂ with the same locality and host, but host eggs collected on 20.II.2002 and adult emergence on 19.VII.2002 (Hérard F. & Cocquempot C.) CIRAD ref. 17884 (in CIRES and Muséum National d'Histoire Naturelle, Paris, France).

Intraspecific variation – The specimens collected in 2002 are somewhat different from the holotype. The main difference concerns the submedian lines of the scutellum which are, in this series, evidently closer to each other, than to the sublateral lines. The specimens are also more lightly colored: the mid lobe of the mesoscutum and the scutellum are brown, more or less reddish, the dorsellum is yellowish as well as the vertex around the ocellar triangle, the occiput on a median line and along the inner edge of eyes; yellowish stripes are present along the adnotaular setae, along the submedian and sublateral lines of scutellum, on the femoral

Table 1 – Pairwise HKY85-parameter distances for D2-28SrDNA sequences (all gaps in the aligned sequences were removed for the purpose of the pairwise analysis). – *aly*, *A. lycidas*. – *sp1*, Costa Rica isolate AJ274451. – *ael*, *A. elongatus*. – *aco*, *A. collega*. – *alu*, *A. luteus*. – *sp2*, UK isolate AJ274452. – *aa*, *A. anoplophorae*. – *sp3*, UK isolate AJ274453. – *hage*, *A. hagenowii* AJ 274454.

	aly01	*aly02*	*sp1*	*ael01*	*ael02*	*aco01*	*aco02*	*alu01*	*sp2*	*aa*	*sp3*	*hage*
aly01	0.0000	0.0000	0.0190	0.0142	0.0142	0.0142	0.0142	0.0094	0.0142	0.0214	0.0364	0.0312
aly02		0.0000	0.0190	0.0142	0.0142	0.0142	0.0142	0.0094	0.0142	0.0214	0.0364	0.0312
sp1[b]			0.0000	0.0287	0.0287	0.0287	0.0287	0.0287	0.0336	0.0410	0.0565	0.0511
ael01				0.0000	0.0000	0.0000	0.0000	0.0047	0.0094	0.0166	0.0314	0.0363
ael02					0.0000	0.0000	0.0000	0.0047	0.0094	0.0166	0.0314	0.0363
aco01						0.0000	0.0000	0.0047	0.0094	0.0166	0.0314	0.0363
aco02							0.0000	0.0047	0.0094	0.0166	0.0314	0.0363
alu01								0.0000	0.0047	0.0118	0.0264	0.0313
sp2[b]									0.0000	0.0166	0.0290	0.0337
aa										0.0000	0.0338	0.0386
sp3[b]											0.0000	0.0463
hage[b]												0.0000

b: species previously published in Genbank.

scrobes, precoxal sutures and submedian stripes on mesopleuron. Also the ovipositor is shorter. These differences might result from a different treatment: those specimens were put into ethanol just after they had emerged, therefore their body might have not been completely sclerotized at that time.

Identification – This species can be recognized by the following combinations of characters: flagellum very long and slender, much longer than width of the mesoscutum; pronotum long, subconical in dorsal view; apical spur of mid tibia short, less than half as long as the basitarsus; gaster acuminate with ovipositor distinctly exserted, combined with the postcercale somewhat shorter than hind tibia; longest cercal seta not kinked. In Graham's key (1987) the species would be keyed out as *Aprostocetus rubicola* on couplet 123 p. 159 because of the very long and slender flagellum of both species. However *A. rubicola* differs in many other respects, especially its much longer epipygium but shorter ovipositor, its scape with 6 setae on the anterior margin, etc. Moreover the hosts are quite different, that of *A. rubicola* is a gall-midge on *Rubus* spp. *Aprostocetus anoplophorae* differs from *A. fukutai* Miwa & Sonan, 1935, and *A. prolixus* LaSalle & Huang, 1994 (LaSalle & Huang 1994) by its much shorter epipygium and exserted part of ovipositor.

Classification – The species clearly belongs to the subgenus *Aprostocetus* and is most probably close to *A. phloeophthori* Graham, 1983, and *A. hedqvisti* Graham, 1987. Both species have long flagellomeres and are also egg-parasitoids of beetles living in dead wood; they were included by Graham (1987) in the large *lycidas* group. From LaSalle & Huang (1994) comments, it seems however also close to *A. fukutai* and to *A. prolixus*, reared from *Apriona germarii* (Hope) (Col., Cerambycidae), in China. Latter species also have long postcercale and ovipositor (still much longer than in all other species of this complex), long flagellomeres, and in *A. prolixus* the longest cercal seta is straight, not kinked. All these egg parasitoids reared from xylophagous beetles probably belong to the same complex of closely related species.

Molecular data

A complete sequence of the D2 region of the 28S rDNA (601 nucleotides) was obtained for the type larva and the adults. Five sequences were deposited in the GenBank database under the accession numbers: *A. anoplophorae* (AY580327), *A. lycidas* (AY580328), *A. luteus* (AY580329), *A. collega* (AY580330), *A. elongatus* (AY580331). The sequences include portions of the 28S rDNA conserved core regions that flanks the D2 region. BlastN alignment searches of the larva sequence data resulted in highest alignment scores with *Melittobia digitata* Dahms, 1984, *M. australica* Girault, 1912, *Aprostocetus* sp., *Cirrospilus* sp., and *Tetrastichus* sp. but not with *Euderus caudatus* Thomson. Alignment with the only four sequences of *Aprostocetus* deposited in GenBank resulted in the analysis of 432 characters after pruning 169 bp on the larva and 169 or 170 bp on the adult sequences that were not present in the GenBank sequences used. Table 1 shows pairwise HKY85 parameter distances for all pairs of the 11 D2-28S rDNA sequences. Except for *A. luteus*, two individuals for each *Aprostocetus* populations collected within the framework

of this research were sequenced. It was not the purpose of this analysis to propose an *Aprostocetus* phylogeny but merely to show the similarity of the sequences to one another as well as some that are already published.

HKY85 distances for D2-28SrDNA sequences ranged from 0 to 0.0565. The levels of differentiation between *A. anoplophorae* and *A. (Tetrastichodes) hagenowii* (Ratzeburg 1852), *A.* sp. 3 from UK, and from *A.* sp. 1, a Costa Rican isolate, varied between 0.0338 to 0.0410. These levels were higher than those obtained with *A. luteus*, *A. lycidas*, *A. elongatus* and *A. collega* which only ranged from 0.0118 (*A. luteus*) to 0.0214 (*A. lycidas*). There was no DNA differentiation observed between *A. elongatus* and *A. collega* specimens as well as within each of the three species (*A. lycidas*, *A. elongatus*, and *A. collega*).

Discussion

The molecular analysis described here provided important clues about the identification of the larva extracted from the egg of *Anoplophora chinensis*. The D2 28S-rDNA sequence data revealed that the larva was unlikely to be related to *E. caudatus*, as it was hypothesized at first on assumptions based upon both the biology of the parasitoid, and its ecological niche. The analysis showed that this taxon might be assigned to another genus, such as *Aprostocetus*. Later on, when adult specimens of this taxon were found, this assignment to the genus *Aprostocetus* was confirmed by comparative morphology analysis. Sequencing data which were also performed on these samples corroborated the above referenced result (data not shown). There was no sequence divergence observed between the samples. A question remained as to what degree this taxon could be part of a new association with *Anoplophora chinensis*, or originated from Asian *A. chinensis*. Answering this question would ideally require a full set of sequences of the *Aprostocetus* species complex. In the absence of Asian specimens to be compared, and of well resolved phylogeny of the *Aprostocetus* species complex, the comparison based on molecular markers with species possibly inclined to parasitize *Anoplophora chinensis* in this part of Europe, was considered meaningful. From the picture resulting from the analysis, we found no evidence that this taxon could be assigned to one of these species. Also, in *Aprostocetus*, not all species can be differentiated using this molecular region; this is the case for example for *A. elongatus* and *A. collega*. Decision of species status, however, cannot be based solely upon genetic distances. The central question remains as to what degree of difference between even similar rDNA markers, is indicative of a species status. In each particular taxonomic group, this level is different and corresponds to its own rate of molecular evolution (Hillis *et al*. 1996; Heraty 2003). However, based upon morphological characters, *A. anoplophorae* is a distinct species within the genus *Aprostocetus*.

It seems to us that the native area of *A. anoplophorae* is more likely in Asia than in Europe. Two main reasons led to this conclusion: During the preliminary specificity test females of the parasitoid appeared to be specific to *Anoplophora chinensis* (Hérard pers. com.). In addition, field observations in Italy showed that the phenology of the parasitoid is quite synchronous with that of its host (Hérard pers. com.). It is very unlikely that a parasitoid from Europe would be able to adapt so quickly to an exotic host. On the other hand, it is assumed that the origin area of *Aprostocetus anoplophorae* in Asia could be Japan rather than China. Several arguments support this hypothesis: The host, *Anoplophora chinensis*, escaped from apple bonsais imported in Italy from the Far East; very likely the parasitoid was present in its host eggs inserted under the bark of the plants. The true origin of the infested plants has never been confirmed by the initial importer. According to other professionals of bonsai trade, the potteries associated with the infested plants are of Japanese style. In Japan, *Anoplophora chinensis* is a common pest in orchards, especially on *Citrus* spp. (Adachi 1994). In southern China, and Taiwan, *Aprostocetus fukutai* is the only egg parasitoid of *Anoplophora chinensis* reported so far. Finding another egg parasitoid species, distinct from *Aprostocetus fukutai*, led us to assume that the host might have originated from Japan.

During several years, concern has been expressed over the need and importance of accurate identification of natural enemies prior to starting any biological control program. This is because of the differences in morphology between pests that are potentially threats to agriculture, and non-pest species; or, even endangered species, can be subtle (Heraty 2003). In a sense, this study serves to illustrate this concern. Insofar as the taxonomic status of the candidate for biological control of CLB has been assured, it is therefore worth considering to proceed to the evaluation of this natural enemy.

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