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A reevaluation of the generic limits of *Pnigalio* Schrank (Hymenoptera: Eulophidae) based on molecular and morphological evidence

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Abstract

Ratzeburgiola Erdös is a small but phylogenetically interesting genus exhibiting a combination of characters once used to define both the former tribes Elachertini and Eulophini of the subfamily Eulophinae (Hymenoptera: Chalcidoidea: Eulophidae). It was once classified in the tribe Elachertini with genera having scutellar grooves and complete notauli, but in some characters it resembles the genus *Pnigalio* Schrank from the former tribe Eulophini. As part of a revision of *Pnigalio* we investigated the phylogenetic relationships between *Ratzeburgiola* and *Pnigalio* using both morphological and molecular data. Based on our results we synonymize *Ratzeburgiola* under *Pnigalio* **n. syn.**, transfer its two included species to *Pnigalio* as *P. cristatus* (Ratzeburg) **comb. n.** and *P. incompletus* (Bouček) **comb. n.**, and reassess the phylogenetic and taxonomic value of morphological characters traditionally considered important at the genus level.

Key words: Chalcidoidea, Eulophinae, *Ratzeburgiola*, phylogeny, synonymy, taxonomy

Introduction

Ratzeburgiola Erdös, 1958 (Hymenoptera: Eulophidae) is a small genus erected for a remarkable species that showed affinities with both the subfamilies Elachertinae and Eulophinae. It currently consists of the type species, *R. cristata* (Ratzeburg), and *R. incompleta* Bouček in Europe, but based on our study there is a third, undescribed species in the Nearctic region. Erdös (1958) considered that the species he designated as the type species of his new genus was the same as *Entedon cristatus* Ratzeburg (1848), though his identification was questioned by Askew (1967, 1968). The type of *E. cristatus* is lost and Thomson (1878) had previously transferred the name to *Elachertus* Spinola and synonymized it with *Elachertus inunctus* Nees. According to Bouček and Askew (1968) the synonymy of *Entedon cristatus* with *Elachertus inunctus* cannot be excluded, but in any case *E. cristatus sensu* Erdös is different from *E. inunctus*.

Ratzeburgiola cristata possesses complete notauli (notaular or parapsidal furrows in Bouček 1959 and 1969, see Gibson 1985 and Menke 1993 for a comprehensive discussion of the taxonomic confusion regarding this morphological feature), which was once used as justification for placing *Ratzeburgiola* in the Elachertini along with other eulophines having complete notauli. However, Erdös' description (1958) of *R. cristata* states that the first three funicular segments of the male antennae are branched, which is a trait more commonly found in Eulophini *sensu* Bouček (1988).

Erdös (1958) considered *Ratzeburgiola* to be very close to *Elachertus*, and therefore placed it in the tribe Elachertini. Bouček and Askew (1968) stated that *Ratzeburgiola* "is an intergrade between Elachertinae and Eulophinae," but Askew (1968) keyed *Ratzeburgiola* within genera of Elachertinae, which was considered a separate subfamily at the time. Bouček (1988) reduced Elachertinae to tribal status within Eulophinae, citing a

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lack of solid character differences between the resulting tribes Elachertini and Eulophini. Subsequently, Gauthier *et al*. (2000) synonymized Elachertini under Eulophini based on the lack of molecular or morphological differentiation between the two groups. These changes reflect a lack of confidence in the value of completeness of notauli as a character for defining suprageneric groups of Eulophinae.

Bouček (1959), Schauff *et al*. (1998) and Burks (2003) considered that *Ratzeburgiola* is near *Pnigalio* Schrank. The propodeum of *R. cristata* is very like that of species of *Pnigalio* in having transverse costulae extending between well-developed median carina and plicae. However, it was not clear from morphological data whether *Ratzeburgiola* is the sister group of *Pnigalio* or whether it comprises a lineage within *Pnigalio* that happened to retain deep and/or complete notauli and therefore renders *Pnigalio* paraphyletic. As part of an ongoing revision of *Pnigalio*, the aim of this paper is to investigate the phylogenetic relationship between *Ratzeburgiola* and *Pnigalio* using morphological observations and three different molecular markers, COI, 28S-D2 and ITS2, which have proven useful at the genus and species level in Eulophidae (Gauthier *et al*. 2000; Gumovsky 2002; Schmidt & Polaszek 2007; Sha *et al*. 2007; Bernardo *et al*. 2008; Li *et al*. 2008; Gebiola *et al*. 2009).

Materials and methods

Specimens were collected between 2006 and 2008 in Italy and the United States. The latter specimens represent the first specimens conforming morphologically to *Ratzeburgiola* collected in the Nearctic region. Host-plants, collection dates, and localities with geographical coordinates are listed in Table 1.

Morphological analysis

Ratzeburgiola specimens used in this study for molecular investigations were compared with specimens identified as this genus at the Natural History Museum (NHM) in London and at the Dipartimento di Entomologia e Zoologia Agraria “Filippo Silvestri”, Università degli Studi di Napoli “Federico II” (DEZA) collection, and with specimens of *Pnigalio* used for molecular analyses. After DNA extraction, specimens from which DNA was extracted were treated as described in Gebiola *et al*. (2009) and card mounted. Voucher material is deposited in DEZA. Morphological characters traditionally used to distinguish between *Pnigalio* and *Ratzeburgiola* (Boucek 1959, 1969; Schauff *et al*. 1998) and other characters studied by Rizzo (1999) were reevaluated based on a large number of individuals. Specimens of *R. cristata* and *R. incompleta* at NHM were all identified by Zdenek Bouček, and labeled as follows:

R. cristata: 3 ♀♀: Spain Granada Otivar 25.VI.1973, Z. Bouček BM 1973-312; 1 ♀: Spain Santander Castro Urdiales 2.VII.1973, Z. Bouček BM 1973-312; 1 ♀: Spain Murcia: Sra de Espana nr Totana 20.6.1973, Z. Bouček BM 1975; 1 ♀: France Var. Saint Tropez 19.VI.80, Z. Bouček BM 1980; 1 ♀: Korfu: Nissaki 6.9.87 J.S. Noyes, Z. Bouček BM 1980; 1 ♂: Spain Murcia: Sra de Espana nr Totana 20.6.1973, Z. Bouček BM 1975 1073-312.

R. incompleta: 2 ♀♀: Cyprus Nikosia ex *Liriomyza* 1988 Min. Ag. N.R., Sp. 2309 CIE A19549; 10 ♀: Israel: Newe ya’ar ex *Lit. blancardella* 1973, H.N. Plant; 1 ♀: Calella d. Costa (Barcel.) Spain VI-1971, Bouček 1977; 1 ♀: Spain Santander Castro Urdiales 2.VII.1973, Z. Bouček BM 1973-312; 1 &: Pakistan C.I.B.C. 12.III.71 Rawalpindi 34, ex larva mining leaves of *S. oleraceno* C.I.E. A469908, *Elachertus* sp. near *isadas* Walk., G.J. Kerrich det 1971; 8 &&: Israel: Sarida Mishmar Haemek 1972 Plant, ex *Lithocoll. blancardella*.

Ratzeburgiola specimens at DEZA were all identified by Gennaro Viggiani and labeled as follows:

R. cristata: 2 ♀♀ ex *Lithocolletis millierella* Stgn. ISRAEL 1/7/69, Coll. Helperin; 1 ♀, ex *C. pulchrimella* on *P. diffusa* Favignana (TP) 11/5/97.

R. incompleta: 3 ♀♀ ex *Lithocolletis* spp. Coltise platany ISRAEL Helperin, 1971.

TABLE 1. Specimens used in this study, with host-plant systems, collection localities and dates, geographical coordinates and GenBank accession numbers.

CODE	SPECIES	SEX	HOST	PLANT	LOCALITY	COORDINATES	DATE	GENBANK ACCESSIONS		
								COI	**28S**	**ITS2**
RC_CS1	*Ratzeburgiola cristata*	♂	*Chrysoesthia sexguttella*	*Chenopodium album*	Sessa Aurunca, Italy	4114’N; 1355’E	10 October 2006	GU361558	GU361542	GU361574
RC_CS2		♂						GU361559	GU361543	GU361575
RC_CS3		♀			S. Giorgio a Cremano, Italy	4049’N; 1420’E	24 September 2006	GU361560	GU361544	GU361576
RC_TC1		♀	*Trachys coruscus*	*Malva* sp.	Cariati, Italy	3920’N; 1655’E	25 April 2007	GU361561	GU361545	GU361577
RI_CS1	*Ratzeburgiola incompleta*	♀	*Chrysoesthia sexguttella*	*Chenopodium album*	Milazzo, Italy	3814’N; 1514’E	8 July 2008	GU361562	GU361546	GU361578
RI_CP1		♂	*Cosmopterix pulchrimella*	*Parietaria diffusa*	S. Giorgio a Cremano, Italy	4049’N; 1420’E	24 September 2006	GU361563	GU361547	GU361579
RI_CP2		♂						GU361564	GU361548	GU361580
RI_CP3		♀			Portici, Italy	4048’N; 1421’E	11 June 2006	GU361565	GU361549	GU361581
RI_TC1		♂	*Trachys coruscus*	*Malva* sp.	Cariati, Italy	3920’N; 1655’E	25 April 2007	GU361566	GU361550	GU361582
RI_TC2		♂						GU361567	GU361551	GU361583
RX1	*Ratzeburgiola* n. sp.	♀	*Agromyza* sp.		Riverside, CA, USA	3358’N; 17719’W	26 August 2008	GU361568	GU361552	GU361584
RX2		♂						GU361569	GU361553	GU361585
PA_BO3	*Pnigalio mediterraneus*	♀	*Bactrocera oleae*	*Olea europaea*	Licusati, Italy	4003’N; 1521’E	3 October 2007	FJ812251	FJ812209	GU361586
PA_BO4		♀			S.Giuliano Terme, Italy	4374’N; 1047’E	23 October 2007	FJ812252	FJ812210	GU361587
PA_CO1		♂	*Cameraria ohridella*	*Aesculus hyppocastanum*	Albenga, Italy	4404’N; 813’E	4 July 2006	FJ812256	FJ812220	GU361588
PA_CO3		♀			Cividale del Friuli, Italy	4605’N; 1326’E	23 November 2007	FJ812258	FJ812222	GU361589
PA_TE2	*Pnigalio agraules*	♀	*Tischeria ekebladella*	*Quercus robur*	Baldissero, Italy	4504’N; 747’E	12 November 2005	FJ812269	FJ812233	GU361590
PA_TE4		♀		*Castanea sativa*	Cumiana, Italy	4459’N; 723’E	4 November 2007	FJ812272	FJ812236	GU361591
PS_HR2	*Pnigalio soemius* complex	♂	*Holocacista rivillei*	*Vitis vinifera*	Latina, Italy	4128’N; 1245’E	27 July 2005	EF507495	FJ812243	GU361592
PS_HR8		♂						EF507501	FJ812244	GU361593
PS_CP1		♀	*Cosmopterix pulchrimella*	*Parietaria diffusa*	Portici, Italy	4048’N; 1421’E	9 June 2006	EF750480	FJ812242	GU361594
PS_CP2		♀			Capua, Italy	4106’N; 1414’E	22 June 2006	EF507486	FJ812241	GU361595
PV_RA1	*Pnigalio vidanoi*	♂	*Rhynchaenus alni*	*Ulmus minor*	Villar Dora, Italy	4506’N; 722’E	7 May 2006	GU361570	GU361554	GU361596
PV_RA2		♀						GU361571	GU361555	GU361597
PV_AP1		♀	*Anthonomus pomorum*	*Malus domestica*	Cumiana, Italy	4459’N; 723’E	23 April 2008	GU361572	GU361556	GU361598
NT1	*Necremnus tidius*	♀	*Cosmopterix pulchrimella*	*Parietaria diffusa*	Portici, Italy	4048’N; 1421’E	11 June 2006	FJ812277	FJ812245	GU361599
EL1	*Elachertus* sp. (*inunctus* group)	♀						FJ812280	FJ812248	GU361600
SS1	*Sympiesis sericeicornis*	♀	*Phyllonorycter millierella*	*Celtis australis*	Portici, Italy	4048’N; 1421’E	23 November 2006	FJ812278	FJ812246	GU361601
DIG1	*Diglyphus* sp.	♀	*Agromyza* sp.	*Silene* sp.	S. Martino Valle Caudina, Italy	4101’N; 1440’E	13 September 2008	GU361573	GU361557	GU361602

Molecular analysis

DNA was extracted from single, whole specimens as described in Gebiola *et al*. (2009). COI and 28S-D2 were amplified as described in Gebiola *et al*. (2009). ITS2 was PCR amplified with primers ITS2F (Campbell *et al.* 1993) and ITS2rev-Trich (Stouthamer *et al.* 1999), which amplify part of the conserved 5.8S at the 5' end and 28S at the 3' end. The thermocycler conditions were: denaturation at 95°C for 3 min, 29 cycles of 94°C for 45 sec, 55°C for 45 sec, 72°C for 1 min, and a last elongation at 72°C for 7 min.

PCR products were checked on a 1.2% agarose gel stained with ethidium bromide, purified using the GeneClean® Kit (MP Biomedicals), and direct sequenced in both directions with an ABI Prism Dye Terminator cycle sequencing kit (Perkin Elmer) and an ABI Prism 310 Genetic Analyzer (Applied Biosystems) sequencer. Nucleotide sequences were deposited in GenBank, with the accession numbers reported in Table 1. Contigs were assembled with Bioedit 7.0 software package (Hall 1999). The alignment of 28S-D2 sequences was based on the RNA secondary structure model developed by Gillespie *et al.* (2005). ITS2 sequences were aligned using the software MAFFT (Katoh *et al*. 2002), and annotated using the ITS2 database (Keller *et al*. 2009).

Diglyphus sp. (Eulophinae: Cirrospilini) was used as outgroup to root the tree. *Sympiesis sericeicornis* Nees, *Necremnus tidius* (Walker), and *Elachertus* sp. (*inunctus* group) (Eulophinae: Eulophini) were also used for the phylogenetic analyses, as well as two species of the *Pnigalio soemius* (Walker) complex *sensu* Bernardo *et al.* (2008) plus *P. vidanoi* Navone, *P. agraules* (Walker) and *P. mediterraneus* Ferrière & Delucchi.

The partition homogeneity test (Farris *et al.* 1994) as implemented in PAUP* 4.0b10 (Swofford 2002), was used to determine the validity of combining mitochondrial and ribosomal genes into a single analysis. One thousand replicates were performed, with 100 random addition sequence replicates and one tree held for each replicate. A resulting P value < 0.01 was considered significant. Phylogenetic reconstructions using Bayesian and maximum likelihood (ML) methods were performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and RAxML (Stamatakis 2006), respectively. MrModeltest 2 (Nylander 2004) was used to select one of the 24 models of nucleotide substitution implemented by MrBayes best fitting the dataset using both the Akaike Information Criterion (AIC) and the four different hierarchies the software uses by default for the likelihood ratio tests (hLRTs) (Posada & Crandall 2001). Two parallel runs of four simultaneous Monte Carlo Markov chains (three heated and one cold) were run in MrBayes for 1×10^6 generations to allow for adequate time of convergence. Trees were sampled every 100 generations for a total of 10,000 trees. Convergence was determined when the average standard deviation of split frequencies dropped below 0.01. The first 2000 trees were discarded as the burn-in, and the phylogeny and posterior probabilities were estimated from a majority-rule consensus of the remaining sampled trees. Bayesian posterior probabilities > 95% were considered to indicate significant support. The ML trees were inferred with RAxML performing 1000 multiple inferences on the original alignment by using the GTRCAT nucleotide model (GTR approximation with optimization of individual per-site substitution rates) starting from a random maximum parsimony tree and initial rearrangement setting and number of rate categories as default. Branch support was evaluated by performing 1000 bootstrap replicates.

Results

Morphological analysis

Morphological observation showed great intraspecific variability in scutellar sculpture in *R. incompleta*, ranging from entirely smooth to almost entirely reticulate. The mesoscutum, axillae and the metascutellum (dorsellum in previous literature) were always smooth in both species of *Ratzeburgiola* (Fig. 1), whereas in *Pnigalio* species examined these areas were always at least shallowly reticulate. The number of costulae on the propodeum of *Ratzeburgiola* species ranged from zero to two. Notauli in *Pnigalio* reached at most half the length of the mesoscutum, whereas the notauli were always more than half the mesoscutal length in *R. incompleta* and complete in *R. cristata* (Fig. 1). The mesoscutal setae in *Pnigalio* ranged from few to many, but they were never as dense, long, or thick as in *Ratzeburgiola* (Fig. 1). Two specimens from the Nearctic region (samples RX1 and RX2) resemble the two described species of *Ratzeburgiola* in having scutellar grooves (though less evident) on a slightly reticulate scutellum as well as having the axillae and metascutellum smooth and the mesoscutum densely setose. The main characters that distinguish these two specimens from *R. cristata* and *R. incompleta* are the length (short versus long) and depth (shallow versus deep) of notauli, whose states resemble those observed in *Pnigalio*. Broad variation in the form of the anterior margin of the scutellum was observed, with different specimens of both *R. cristata* and *R. incompleta* having the margin either straight or convex. Lastly, *Ratzeburgiola* species share a number of morphological synapomorphies with the *Pnigalio soemius* species complex *sensu* Bernardo *et al*. (2008): one to three always long paraspiracular propodeal setae, small fore wing speculum, gaster at least partly yellow or brown instead of metallic as in *P. agraules* and *P. mediterraneus*.

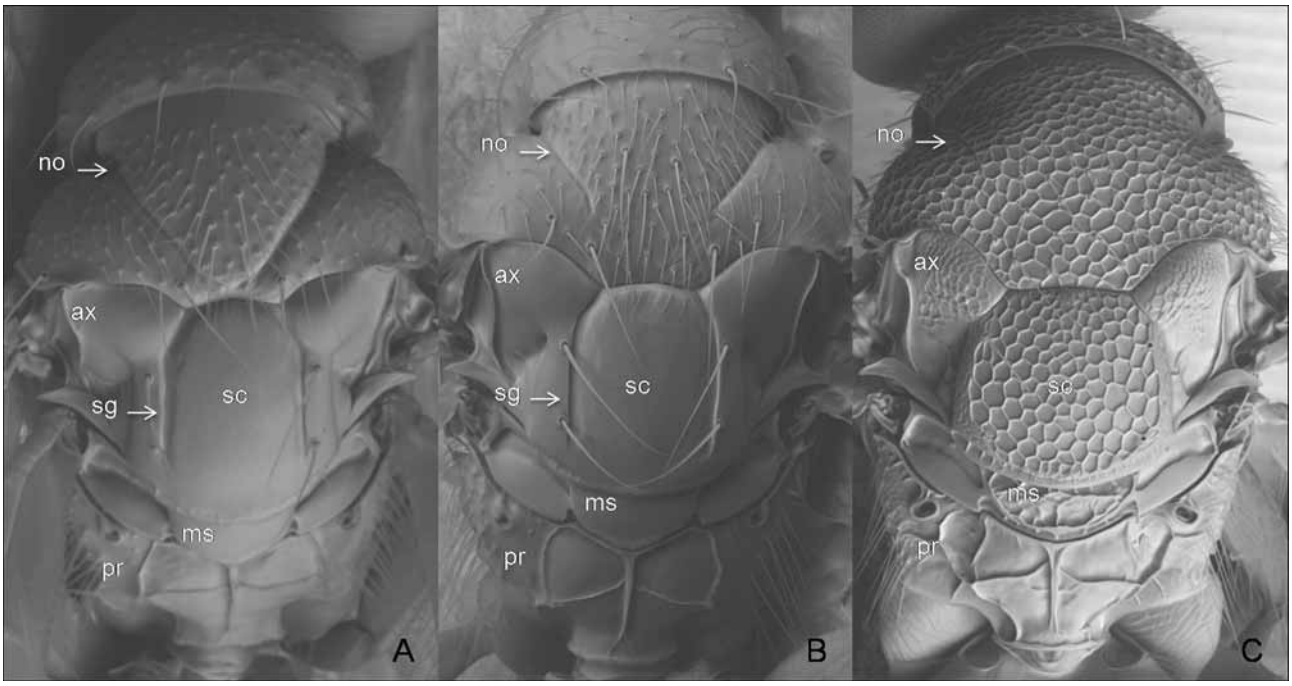

FIGURE 1. Mesosoma of *Ratzeburgiola cristata* (A), *R. incompleta* (B), *Pnigalio vidanoi* (C) females: ax, axillae; no, notauli; pr, propodeum; ms, metascutellum; sc, scutellum; sg, scutellar grooves.

Molecular analysis

Trimmed COI sequences were 688 bp, and the alignment was straightforward; no frame shifts, nonsense codons, insertions or deletions were identified in any sequence. 28S-D2 sequences ranged from 427 to 439 nucleotides, and the final alignment consisted of 444 bp. The secondary structure model allowed identification of a region variable both in nucleotide composition and in sequence length in the compound helix 3–1 (helices 3a–3f–3), making the alignment unambiguous. Both Palaearctic *Ratzeburgiola* species and Nearctic specimens RX1 and RX2 share the same 28S-D2 sequence with the two species of the *P. soemius* complex. This shared sequence is unusual in Chalcidoidea in lacking D2 variation (Gebiola *et al*. in prep.). ITS2 sequences ranged from 386 to 501 nucleotides, and the final alignment consisted of 650 bp. The p-value for the partition homogeneity test was 0.79, indicating that the three gene datasets were not incongruent and could be combined into a single dataset and analyzed simultaneously. Therefore, the final concatenated alignment consisted of 1782 bp.

MrModeltest consistently selected the GTR+I+G substitution model, corresponding to the following commands in MrBayes: Lset nst = 6 rates = invgamma; Prset statefreqpr = dirichlet (1,1,1,1). The Bayesian consensus tree (Fig. 2) shows that *Pnigalio* and *Ratzeburgiola* form a fully supported monophyletic assemblage (posterior probability = 1.00). The best-scoring ML tree (likelihood = -7872.523629) has a similar topology (Fig. 3), with monophyly of *Pnigalio* + *Ratzeburgiola* again well supported (bootstrap = 97). In both trees Palaearctic and Nearctic *Ratzeburgiola* species are paraphyletic. Neither analysis resolved outgroup relationships, although *Elachertus* appears not to be closely related to *Ratzeburgiola*. Furthermore, within *R. incompleta*, specimens reared from three different hosts *Chrysoesthia sexguttella* (Thunberg), *Trachys coruscus* (Ponze) and *Cosmopterix pulchrimella* Chambers formed three different clades.

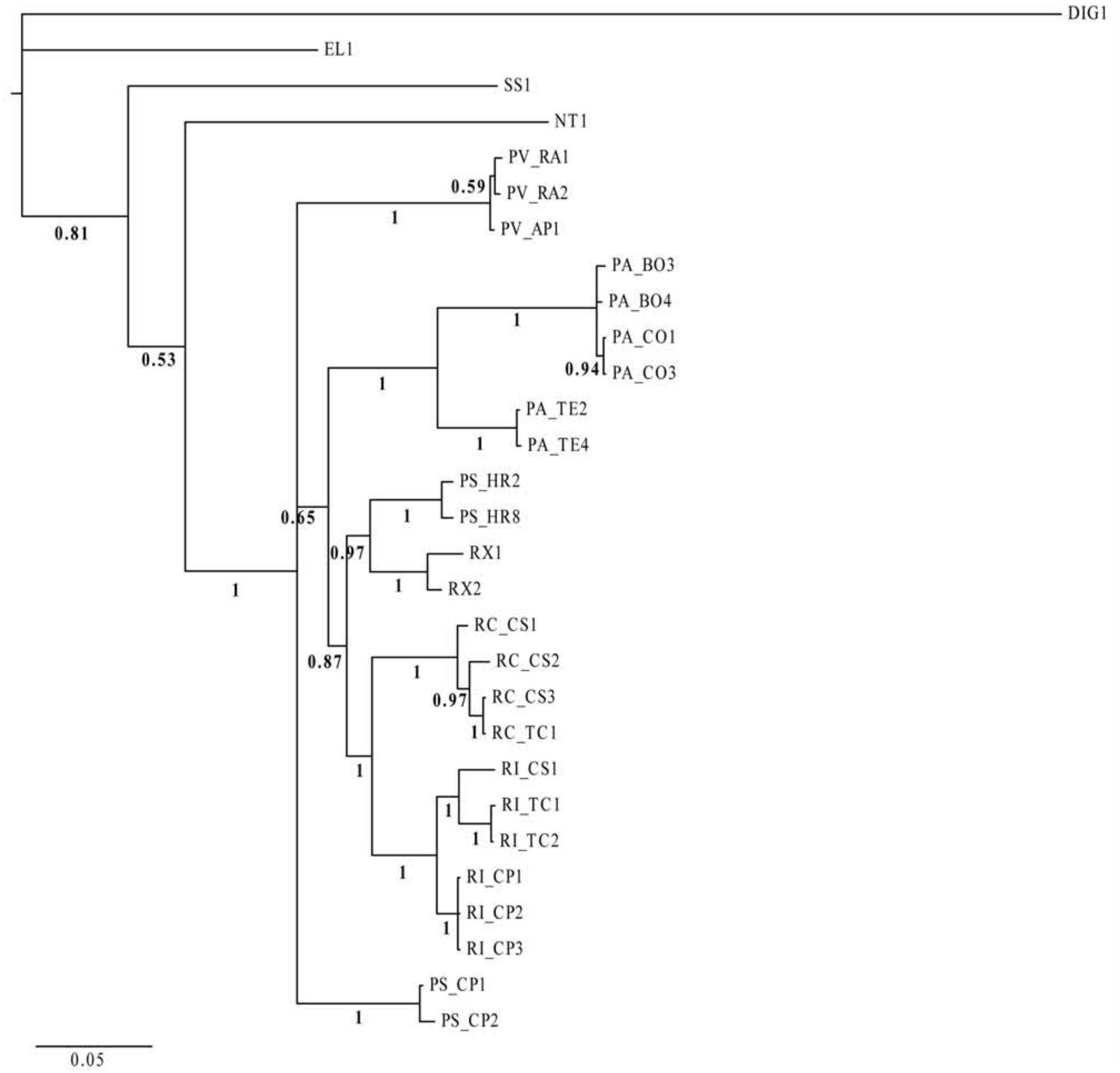

FIGURE 2. Majority rule consensus Bayesian tree based on a concatenated dataset of 28S-D2, ITS2 and COI sequences. Posterior probability values are indicated in bold below nodes. The species identity for each taxon code is given in Table 1.

Discussion

Species assigned to *Ratzeburgiola* resemble those classified in *Pnigalio* by having a propodeum with complete plicae and transverse costulae (Fig. 1), though the number and shape of the latter vary as they do in *P. soemius* (see Bernardo *et al*. 2007) but differ by having some other traits not found in *Pnigalio*, such as the presence of scutellar grooves, complete notauli (in *R. cristata*), smooth axillae and metascutellum, and a more densely setose mesoscutum (Fig. 1). However, our observations show that many of the morphological characters of *Ratzeburgiola* are quite variable, sometimes more than previously recorded, as has also been shown for several species of *Pnigalio* by Bernardo *et al*. (2007, 2008) and Gebiola *et al*. (2009). For example, we found that specimens of *R. incompleta* identified by Z. Bouček had the scutellum completely smooth and shiny to moderately sculptured, a variation already recorded by Bouček (1969), yet we never observed a scutellum coarsely punctured-reticulate with the sides smooth. Another similar sculpture pattern, previously reported by Rizzo (1999), was observed also for *R. cristata*. Another very variable character is the number of propodeal costulae, whose variation equals that recorded in several Nearctic species of *Pnigalio* (Barrett *et al*. 1988) and in *P. soemius* (Bernardo *et al*. 2007). Furthermore, the differential character that was highlighted by Rizzo (1999) to distinguish *R. incompleta* and *R. cristata* (shape of the anterior margin of the propodeum, straight in *R. incompleta* and convex in *R. cristata*) does not hold for all specimens we examined. Because of its high

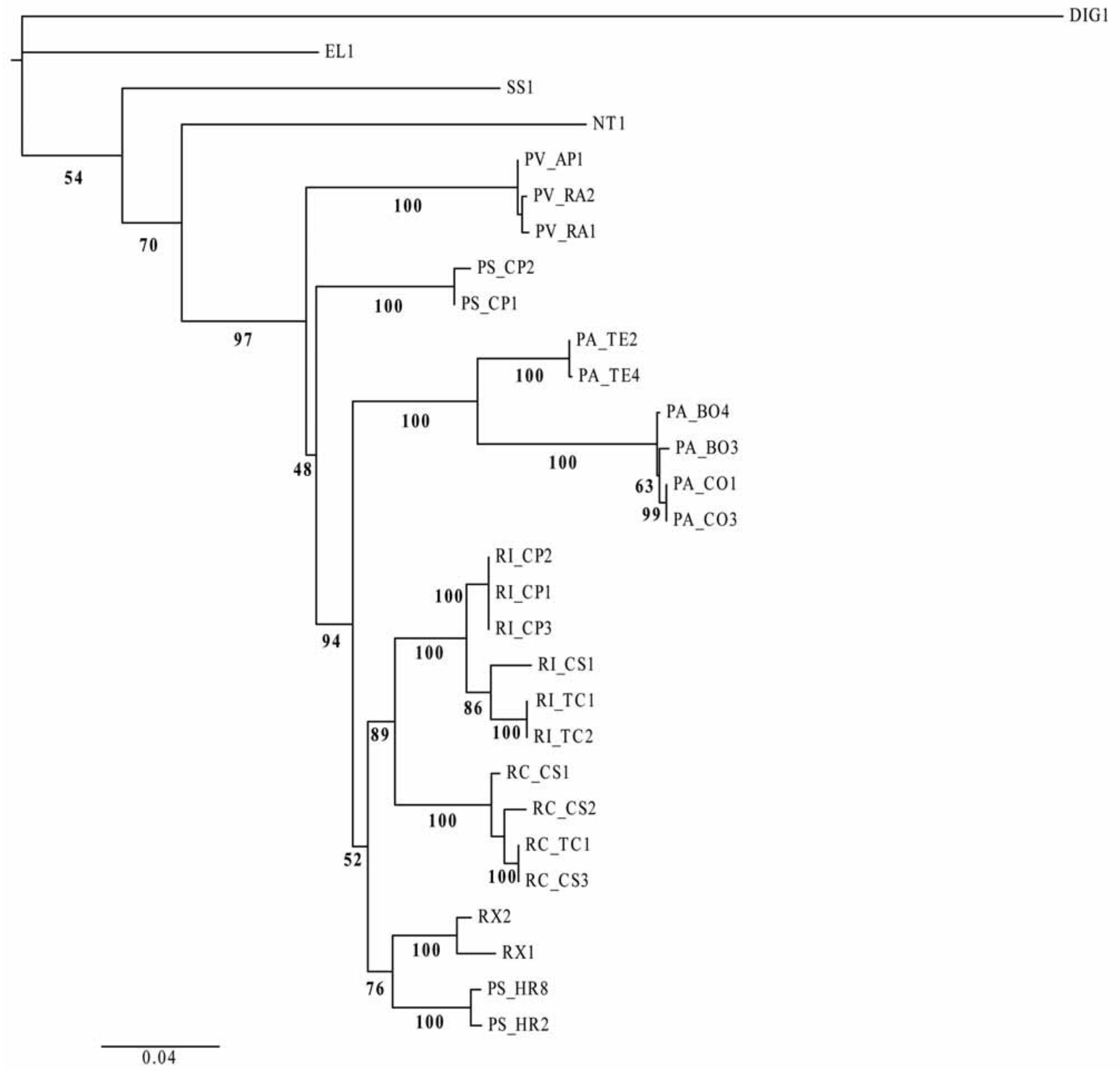

FIGURE 3. Best scoring maximum likelihood tree based on a concatenated dataset of 28S-D2, ITS2 and COI sequences. Bootstrap values are indicated in bold below nodes. The species identity for each taxon code is given in Table 1.

intraspecific variability, this character cannot be considered reliable to distinguish between the two species. Based on our observations, the only reliable character distinguishing the two described species of *Ratzeburgiola* is the length of the notauli, all other characters' states largely overlapping due to high intraspecific variability. In this paper we also report two Nearctic specimens that are referable to the traditional concept of *Ratzeburgiola,* and in these two specimens the notauli are only barely visible, as in most *Pnigalio* species. Additionally, the scutellar grooves are not as evident in the Nearctic specimens as in Palaearctic *Ratzeburgiola*. Our phylogenetic analyses using molecular evidence seem to confirm the morphological observations because the Nearctic specimens do not form a monophyletic group along with the two described species of *Ratzeburgiola*, but are indicated as the sister group of one species of the *P. soemius* complex. The two Nearctic specimens likely represent an undescribed species based on both morphological and molecular data. However, before this new species can be adequately described more individuals are needed to thoroughly address the real extent of intraspecific variation (see Dayrat 2005). Paraphyly of the three *Ratzeburgiola* species would indicate that the scutellar grooves evolved at least twice, but a lack of node support prevents a definitive conclusion.

Some authors (Bouček 1959; Schauff *et al*. 1998; Burks 2003) have argued previously that *Ratzeburgiola* is very close to *Pnigalio*. Our molecular analyses clearly show that *Ratzeburgiola*, falls within *Pnigalio*, regardless of morphological differences outlined above between the three *Ratzeburgiola* species and all *Pnigalio* species here considered. The single 28S-D2 haplotype recovered for the three *Ratzeburgiola* species and the *P. soemius* species complex may be instead regarded as a molecular synapomorphy. For a marker so conserved in *Pnigalio,* even a single mutational difference in 28S rDNA may be taken as an informative character that could potentially delineate a new species group (see Sonnenberg *et al*. 2007). It is also very remarkable that such a unique ribosomal haplotype is shared by species that are geographically isolated (Palaearctic and Nearctic region) and morphologically distinct. Another interesting finding evident from the phylogenetic trees is that within *R. incompleta* there appears to be a tendency toward host specialization, a pattern already observed for the *P. soemius* species complex by Bernardo *et al*. (2008). This needs to be investigated more thoroughly and will be discussed further elsewhere (Gebiola *et al.* in prep.).

Given the molecular evidence and the newly discovered morphological variability in such characters as length of the notauli, number of propodeal costulae and scutellar sculpture of the three known species, it is highly problematic to retain the status of *Ratzeburgiola* as distinct from *Pnigalio*; therefore we synonymize *Ratzeburgiola* under *Pnigalio* **syn. n**. This results in the following new combinations for the two species previously contained in *Ratzeburgiola*: *Pnigalio cristatus* (Ratzeburg) **comb. n.** and *Pnigalio incompletus* (Bouček) **comb. n**.

Based on our study, the importance of the three main characters traditionally used to discriminate between *Pnigalio* and *Ratzeburgiola*, the length and depth of notauli and the scutellar grooves, need to be further questioned as characters of generic or greater significance. The former two characters, once used to distinguish Elachertinae and Eulophinae, were already considered by Bouček (1959) not to have any more major taxonomic importance than other characters. Elachertini was used later by Bouček (1988), but with some doubts about the characters. Gauthier *et al*. (2000) stated that the condition of the notauli (complete or incomplete) is a generally unreliable character. Sha *et al*. (2007) further investigated the notaular evolution in Eulophinae based on molecular data, and proposed that complete curved notauli evolved independently at least five times and incomplete notauli at least four times. Therefore, as stated by these authors, the multiple, independent origin of derived notaular shapes suggests that this character is potentially misinformative, and that notaular patterns may continue to serve as diagnostic characters only when taken in concert with other morphological characters. Our molecular data represent a further strong confirmation of such a statement, demonstrating that the extent of the notauli cannot be used to discriminate between two genera in this case.

Our results also agree with previous doubts about the value of scutellar grooves as a generic grouping character. Among members of former tribe Elachertini, the scutellar grooves are weakly developed or absent in *Paraolinx* Ashmead, and *Miotropis* Thomson is closely related to, or potentially synonymous with, *Elachertus*, but lacks scutellar grooves. *Dicladocerus* Westwood is closely related to *Pnigalio* and other Eulophini, but possesses parallel scutellar grooves. Some Australasian Eulophinae of dubious placement may be closely related to *Sympiesis* Förster, but have scutellar grooves (Burks unpublished). Genera of the subfamily Entedoninae agree with these patterns of variation in that *Horismenus* Walker (Hansson 2009) and *Derostenus* Westwood (Hansson 1986) exhibit polymorphism in scutellar grooves. These observations agree with previous authors that presence of scutellar grooves may be an informative character at some level, but this level may be only that of species or species groups within genera, and not that of the level of tribe or subfamily.

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