Phylogenetic evaluation of the taxonomic status of *Timandra griseata* and *T. comae* (Lepidoptera: Geometridae: Sterrhinae)

**EIRKI ÕUNAP**1,2, JAAN VIIDALEPP2 and URMAS SAARMA1,3

1Department of Integrative Zoology, Institute of Zoology and Hydrobiology, University of Tartu, Vanemuise 46, 51014 Tartu, Estonia; e-mail: erkio@ut.ee; Urmas.Saarma@ut.ee

2Institute of Agricultural and Environmental Sciences, Estonian Agricultural University, Riia 181, 51014 Tartu, Estonia; e-mail: jaan@zbi.ee

3Estonian Biocentre, Riia 23, 51010 Tartu, Estonia

**Key words.** Geometridae, Sterrhinae, *Timandra*, COI, ND1, phylogeny, taxonomy, hybridization

**Abstract.** The sterrhine loopers *Timandra griseata* and *T. comae* have been treated as distinct species since 1994. However, morphological differences between the taxa are minor and therefore their status has often been disputed. Here, we present a molecular phylogenetic study, which separates *T. griseata* and *T. comae* into different clades. Altogether, 43 *Timandra* specimens from eight European countries were studied. The phylogeny is based on a comparative sequence analysis of mitochondrial genes coding for the cytochrome C oxidase subunit I (COI) and NADH dehydrogenase subunit 1 (ND1). Nevertheless, a single individual of both species was assigned to the “wrong” clade. The symplesiomorphy of *T. griseata* and *T. comae* is considered to be a result of introgressive hybridization. Conditions that could lead to the hybridization of *T. griseata* and *T. comae* are discussed, as well as the likely distribution history of these taxa in Northern Europe. Results of the current analysis are in favour of retaining the species status of *T. griseata* and *T. comae*.

**INTRODUCTION**

The sterrhine genus *Timandra* Dupechel, 1829 is distributed mainly in the Eastern Palaearctic and Oriental region (Kaila & Albrecht, 1994). Only three species occur in the Western Palaearctic (Müller, 1996) and one in the Nearctic (Prout, 1935–38). The total number of species in this genus is 21 (Scoble, 1999). Most species in this genus have similar wing pattern, but differences in genitalia enable the species to be distinguished quite easily (Inoue et al., 1982).

Kaila & Albrecht (1994) defined the *Timandra griseata* group as including species that have a short and blunt sacculus and sclerotized costa of the valva branching at three quarters from the base, forming two branches of about equal length. Three species were considered to belong to this group: sibling species *T. griseata* (Petersen, 1902) and *T. comae* (Schmidt, 1931) in the Western Palaearctic and *T. recompta* (Prout, 1930) in the Eastern Palaearctic. Of these species, *T. recompta* clearly differs from the others, especially in the characters of the female genitalia. *T. griseata* and *T. comae* were treated as separate species for the first time by Kaila & Albrecht, 1994, who also used the name *comai*. The name *comai* by Schmidt (1931), which originally was given in honour of Mr. Pedro Coma, was found to be an incorrect original spelling (Kaila & Albrecht, 1994). Their emendation from *comae to comai* was based on the third edition of the Code ICZN (1985), article 31(a)(ii), which states that if a noun in the genitive case is formed directly from a modern personal name of a man, -s has to be added to the stem of that name; see details also in Kullberg et al. (2002). The name *comai* was subsequently used in several publications (eg. Kaila & Albrecht, 1995; Palmqvist, 1997; Kaila et al., 1999; Sihvonen, 2001; Kullberg et al., 2002).

On the other hand, an alternative interpretation is possible, as article 31(a)(i) in Code ICZN (1985) and article 31.1.1. in Code ICZN (1999) state that a noun in the genitive case formed from a personal name that is Latin or has been latinized, is to be formed in accordance with the rules of Latin grammar. Following this paragraph results in *comae* as used in three major recent moth catalogues by Müller (1996), Scoble (1999) and Hausmann (2004). Based on this interpretation and the principle of priority, we consider *comae* to be the valid name and *comai* to be an unnecessary emendation.

Morphological differences between *T. griseata* and *T. comae* are often regarded as insufficient to treat them as separate species. Therefore, their specific status has been questioned by lepidopterists and criticized in literature (Hausmann, 1997, 2004). The external morphology of *T. griseata* and *T. comae* differs as follows: ground colour of the forewing is whitish in *T. griseata*, yellowish in *T. comae*, dusting on wings is dense and grey in *T. griseata*, sparse and brownish-grey in *T. comae*, average wingspan is bigger and sexual size dimorphism more accentuated in *T. griseata* (Kaila & Albrecht, 1994). There are small differences in the position of the junction between the anterior and posterior parts of corpus bursae and in the angle of appendix bursae of the females. The external genitalia of male *T. griseata* and *T. comae*, however, are indistinguishable (Kaila & Albrecht, 1994). On the other hand, a detailed study of everted vesicae by Sihvonen (2001) supports the original hypothesis of Kaila & Albrecht (1994).
In addition to the differences in morphology, the distribution of *T. griseata* and *T. comae* is also different (Kaila & Albrecht, 1994). These species are allopatric over most of their geographic range, with a narrow contact zone in Fennoscandia and the northern part of the Baltic countries. *T. comae* is widely distributed in the Western Palaearctic – its range covers all of western, southern and eastern Europe, reaching southern Finland, central Sweden and southern Norway in the north and Turkmenistan in the east (Müller, 1996; Viidalepp, 1996; Aarvik et al., 2000; Huldén et al., 2000; Hausmann, 2004). *T. griseata* is restricted to northern Europe. This species is widely distributed in Finland and Sweden (Huldén et al., 2000; Hausmann, 2004) and has a wider range in southern Norway than *T. comae* (Aarvik et al., 2000). *T. griseata* has also been recorded in northwestern Russia (Kaila & Albrecht, 1994), but is rare in Estonia and Latvia (Kaila et al., 1999, Savenkov & Šuks, 2004).

The only report of *T. griseata* from Denmark (Larsen, 1995), as well as notes that this species occurs in Belarusia, Ukraine, Crimea, the Ural Mountains, Caucasus and Trans Caucasus (Viidalepp, 1996) have been interpreted as erroneous (Hausmann, 2004). Moreover, *T. griseata* and *T. comae* differ in their phenology. In Finland, where *T. griseata* and *T. comae* are sympatric, the first imagoes of the bivoltine *T. comae* appear about two weeks earlier than the earliest specimens of the usually univoltine *T. griseata*. The first generation of *T. comae* is less abundant than the second, therefore, this species is most numerous in August, while the number of *T. griseata* peaks in late June (Kaila & Albrecht, 1994).

Recent short reports on the differences in mitochondrial NADH dehydrogenase subunit 1 (*ND1*) (Miller et al., 2001) and cytochrome C oxidase subunit I (*COI*) (Trusch et al., 2002) sequences of *T. griseata* and *T. comae* also indicate that these taxa might represent separate species. However, since Miller et al. (2001) used only one specimen of *T. griseata* and two of *T. comae* and Trusch et al. (2002) have not published the details of their study, an additional and more extensive molecular survey is needed to resolve the taxonomic status of *T. griseata* and *T. comae*; the need for further investigation is emphasized also by Hausmann (2004).

In this study, we analysed specimens of *T. griseata* and *T. comae* from Estonia and Finland, where these taxa are sympatric (Kaila & Albrecht, 1994; Müller, 1996; Kaila et al., 1999), and a few additional individuals from other European countries (Latvia, Sweden, Germany, Belorusia, Ukraine and Bulgaria). Partial sequences from mitochondrial *COI* and *ND1* genes were used for phylogenetic inference, since mitochondrial genes are reported to be more useful than nuclear genes in most cases for resolving the boundaries of recently diverged species (Caterino et al., 2000; Wiens & Penkrot, 2002).

**MATERIAL AND METHODS**

**Moths**

Forty-three specimens of the European *T. griseata* group from 27 localities were studied (Table 1). Most of the material (36 individuals) was collected from 20 localities in Estonia and Fin-

---

**Fig. 1.** Sampling localities of *T. griseata* (open circles), *T. comae* (filled circles), and both species (half-filled circles) in Estonia and Finland. Specimens, their numbers and localities are presented in accordance to the Table 1.
A 392-bp fragment was amplified from COI and 398-bp fragment from ND1 gene. PCR was performed in a total volume of 20 μl. The reaction mixture contained 1X BD Advantage 2 PCR buffer, 1U BD Advantage 2 Polymerase mix (BD Biosciences, San Jose, USA), 0.2 mM dNTP (Fermentas, Vilnius, Lithuania); 1.5 mM MgCl₂, 4 pmol of primers and 20–80 ng of purified genomic DNA.

PCR was performed on a T1 Thermocycler (Biometra, Gottingen, Germany), cycling parameters were a 2 min denaturing
TABLE 2. Nucleotide variation in mtDNA COI and ND1 sequences of T. griseata and T. comae. The number of specimens for each haplotype and the number of localities for each haplotype are given in the last two columns. Dots indicate nucleotide identity to the T. comae 24 sequence. Numbering corresponds to the homologous sequence of Bombyx mori (AY048187). Substitution types are indicated as tv for transversion, ts for transition, and * for both transition and transversion.

| Nucleotide position according to B. mori | COI | ND1 |
|----------------------------------------|-----|-----|
| Codon position                         | tv  | ts  |
| T. com 24                              | C   | G   |
| T. com 150                             | A   | G   |
| T. com 228                             | G   | A   |
| T. com 240                             | T   | A   |
| T. com 243                             | G   | A   |
| T. com 244                             | C   | G   |
| T. com 251                             | T   | A   |
| T. gri 82                              | C   | G   |
| T. gri 157                             | A   | G   |
| T. gri 182                             | C   | A   |
| T. gri 183                             | A   | C   |
| T. gri 190                             | G   | A   |
| T. gri 241                             | G   | A   |
| T. gri 254                             | T   | A   |

| Substitution type | COI | ND1 |
|-------------------|-----|-----|
| tv                | G   | G   |
| ts                | A   | G   |
| ts                | G   | A   |
| ts                | A   | G   |
| ts                | C   | G   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | A   | T   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | A   | T   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | A   | T   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | A   | T   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | A   | T   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
| ts                | T   | C   |
| ts                | G   | A   |
| ts                | T   | A   |
transversion rate was 22 : 4. Nineteen substitutions were synonymous and six non-synonymous. Intraspecific variation occurred at six positions (0.76%) in the *T. comae* and 12 positions (1.52%) in *T. griseata* sequences. Five transitions and two transversions (0.89%) distinguished *T. griseata* from *T. comae*. In the COI locus, homoplasy was found at position 591, both A and G was recorded in *T. griseata* individuals, while all *T. comae* specimens carried T at this site.

On a minimum spanning network (Fig. 2), *T. griseata* is clearly separated from *T. comae*, the shortest distance between the closest haplotypes of these species is 10 mutations. *T. comae* form a single haplogroup with seven haplotypes. Haplotype C24 is the central and by far the most numerous one in this haplogroup (21 specimens, all other *T. comae* haplotypes are represented by a single specimen). *T. griseata* can be divided into two haplogroups: the first consists of haplotypes G183, G254 and G182. The second haplogroup, consisting haplotypes G157, G190, G82 and G241, is six mutations away from the haplotype G183. No haplotype of *T. griseata* was significantly more abundant than the others (Fig. 2).

The model (TrN+I+G) was selected by Modeltest according to the Akaike Information Criterion. As it is not possible to implement the TrN+I+G model in MrBayes, the GTR+I+G model was used. Three independent Bayesian analyses converged on statistically equivalent log-likelihood scores and reached an asymptotic level after no more than 20,000 iterations (not shown). Majority rule consensus trees of three rounds of Bayesian analysis were identical and the analyses converged on a single optima. Both neighbour-joining and Bayesian inference gave similar tree topologies, albeit with different statistical support (Fig. 3). A major feature of the phylogenetic analysis, strongly supported by both methods, is that it divides *T. comae* and *T. griseata* into separate clades. *T. griseata* has a more pronounced internal structure, consisting of two subclades. Three of the four individuals in subclade I were collected from northern Estonia (Pakme, Narva-Jõesuu and Endla Nature Reserve), and one from the Tatra Valley near Tartu in eastern Estonia (Fig. 1). Specimens in subclade II originate from a variety of localities in eastern, southwestern and northwestern Estonia and southern Finland (Table 1).

**DISCUSSION**

The phylogenetic analysis separated *T. griseata* and *T. comae* into different clades (Fig. 3), supporting the suggestion that these taxa represent separate species (Kaila & Albrecht, 1994; Miller et al., 2001) or, as expressed by Trusch et al. (2002), “natural entities”. Nonetheless, two individuals, *T. griseata* 180 and *T. comae* 137, were included in the clade of the other species. In order to exclude the possibility of misidentification, the morphological characters of both individuals were re-examined;
our identifications were later confirmed by L. Kaila (University of Helsinki). To rule out mistakes in genetic analysis (picking wrong individual, contamination, sequencing errors) and subsequent misinterpretation of data, the whole analysis from dissection to DNA purification and sequencing was repeated for these two specimens, using the posterior parts of their abdomens as the source of genomic DNA (see also Material and Methods). As this re-examination confirmed the results of the first analysis, we conclude that the identity and positions of *T. griseata* 180 and *T. comae* 137 in the phylogenetic tree are valid.

Following the Mayr’s (1963) biological species concept, it is generally assumed that species must be monophyletic entities (Harrison, 1998). However, as shown by Pamilo & Nei (1988) and summarized by Wahlberg et al. (2003) and Funk & Omland (2003) in various sources, both polyphyley and paraphly may arise during the speciation process. Complete lineage sorting may or may not have occurred during speciation and it cannot be assumed as an ineluctable event (Wahlberg et al., 2003).

Incomplete lineage sorting can result either from a recent divergence of populations, or from hybridization, which does not allow complete segregation of lineages. If complete lineage sorting has occurred at a pre- or postzygotic level (genes associated with genitalia formation, pheromone system, etc.), to explain the divergence of species, less time has elapsed for mutations to become fixed; both species still share identical or very similar haplotypes (i.e. have retained the polymorphism of their ancestral population). However, the claim of incomplete lineage sorting holds only for the genetic markers under investigation, usually the genes of mtDNA. It is conceivable that complete lineage sorting has occurred at other loci in the genome; candidates are primarily genes related to reproduction and the formation of a reproductive barrier between diverged populations, either at a pre- or postzygotic level (genes associated with genitalia formation, pheromone system, etc.). To find the sorted locus or loci for animals other than model organisms is an unrealistic task at present. A statement solely in favour of incomplete lineage sorting between species presumes that hybridization is ruled out. If hybridization occurs and the hybrids yield fertile offspring, then successful backcrossing does not allow lineages to diverge. On the other hand, when diverged populations hybridize (i.e. after the disappearance of geographic barriers), but do not produce fertile offspring, then one can still observe individuals that carry haplotypes of another species. However, since they do not pass genes to the next generation, the parent population retains its genetic status.

Here, the closest mtDNA haplotypes of *T. griseata* and *T. comae* differ from each other by at least 10 substitutions (Fig. 2), indicating that these taxa did not separate from each other recently. Moreover, *T. griseata* has a significantly diverged intraspecific genetic structure (Fig. 3), which supports the hypothesis that intraspecific diversification of *T. griseata* has ancient history. However, it is unlikely that haplotypes of *T. comae* 137 and *T. griseata* 180 have retained their ancestral mtDNA sequence, while a remarkable amount of mutations have accumulated in the population of *T. griseata* (and to lesser extent in *T. comae*). Therefore, we favour the scenario that the few atypical sequences in *T. griseata* and *T. comae* most likely originated from hybridization. Additional support for the hybridization scenario comes from the phenology of *T. griseata* and *T. comae*. *T. comae* is bivoltine, while *T. griseata* is usually univoltine. Both species are protandric (i.e. males appear earlier than females), as most insects with discrete generations (e.g. Carvalho et al., 1998). Flight periods of *T. griseata* and *T. comae* differ, but partly overlap in southern Finland (Kaila & Albrecht, 1994).

In Estonia, a similar pattern was observed (Fig. 4): the flight period of the first generation of *T. comae* ranges from late May to the end of June; the second and more abundant brood is on wing from late July to September. The flight period of *T. griseata* begins in late June and peaks in the middle of July, lasting until the end of the month. Therefore, the flight periods of the species overlap at least twice in each year, providing an opportunity for the species to hybridize. The earliest possible time when hybridization can occur is the end of June, when both sexes of the first generation of *T. comae* and only males of *T. griseata* are flying, but females of *T. griseata* have not emerged yet. Males of *T. griseata* not finding a receptive female of their own species can copulate with females of *T. comae*.

Hybridization may also occur at the end of July, when both sexes of *T. griseata*, but only males of the second generation of *T. comae*, are on wing and females of the second *T. comae* brood have not yet emerged. At this time, males of *T. comae* may hybridize with females of *T. griseata*. However, it is unlikely that there are many unfertilized females of either *Timandra* species searching for mates at the end of their flight period. Therefore the probability for hybridization is low, which can explain why only two *Timandra* specimens of possible hybrid origin were found during this study.

The hypothesis of hybridization between *T. griseata* and *T. comae* receives further support from the discussion of Pittaway (1993) for closely related sphingid species *Hyles euphorbiae euphorbiae* (Linnaeus, 1758) and *Hyles*.
vespertilio (Esper, 1780). The flight period of *H. vespertilio* starts 8–14 days earlier than that of *H. e. euphorbiae*. When the earliest *H. e. euphorbiae* males emerge, there are no receptive conspecific females available for them; therefore, they sometimes hybridize with females of *H. vespertilio*. Although *H. e. euphorbiae × H. vespertilio* is the commonest sphingid hybrid in Europe, the parent species have remained distinct, because hybrids are unable to produce viable offspring when back-crossed with *H. vespertilio* (Pittaway, 1993). However, while *H. e. euphorbiae* and *H. vespertilio* are morphologically rather different and hybrids are easily distinguishable, *T. comae* 137 and *T. griseata* 180 show no intermediate morphological characteristics usually seen in F1 generation hybrid sphingids. A likely explanation is that *T. comae* 137 and *T. griseata* 180 are not F1 hybrids and the hybridization event is more ancient.

The eastern limit of *T. griseata* is unknown, but it is probably significantly further east than current data indicate. Notes that *T. griseata* occurs south of Scandinavia and the Baltic States (Viidallev, 1996) should be interpreted with care until validated (as the identification was carried out before the recognition of *T. griseata* and *T. comae* as separate species, all individuals were identified as *T. griseata*).

*T. griseata* and *T. comae* became sympatric during the 20th century. *T. griseata* has been resident in Finland since the 19th century (the oldest records date back to 1869), while *T. comae* expanded its distribution into this area during the 20th century. The earliest Finnish specimen of *T. comae* was taken in 1920 (Kaisila, 1954; Kaila & Albrecht, 1994). Our survey of the Estonian insect collections gave similar results: all *Timandra* individuals, collected between 1874 and 1939, were identified as *T. griseata*; the earliest Estonian *T. comae* specimen was collected in 1940. The largely different geographic distributions and timing of colonization by these species supports Kaisila’s hypothesis, that *T. griseata* (*Calothyssanis amatoria griseata* in his paper) and *T. comae* (*C. a. brykaria*) have different colonization histories in northern Europe: in addition to the differences in their time of arrival, they also came via different routes: *T. griseata* from the east and *T. comae* from the west.

The *T. griseata* clade is divided into two subclades (Fig. 3) that differ in geographic distribution. Most of the specimens from Estonia and Finland belong to the subclade II, but three haplotypes constitute the subclade I. Three specimens of the subclade I were from northern Estonia (i.e. all north Estonian individuals) and one from the Tatra Valley near Tartu in eastern Estonia (Fig. 1). Data on the phylogenetic structure and geographic distribution of the different haplotypes of *T. griseata* further indicate that this species may have come from two directions. The most likely scenario is that specimens of subclade I spread into Estonia via the northern side of Lake Peipsi, while ancestors of subclade II came via a southern route (Fig. 5). A similar colonization pattern is documented for another Lepidopteran, *Parnassius mnemosyne estonicus* Bryk 1922, which rapidly expanded its range, both in northern and southeastern Estonia, during the last two decades (Viidallev, 2000).

The completely different intraspecific genetic structure of *T. griseata* (highly divergent, ancient radiation) compared to *T. comae* (limited divergence, rapid radiation) proves that the evolution of these taxa has been different. The complex phylogenetic structure and current geographic distribution of *T. griseata* in northern Europe indicates that this species colonized northern Europe from several (or at least two) refugia. An alternative possibility, that the population with high intraspecific genetic polymorphism came from a single refuge area, is less likely because of its current distribution pattern. Limited genetic divergence of the population of *T. comae* suggests that all current populations in Europe came from a single refuge area and descend from a population with a low polymorphism at the mtDNA loci. Another explanation that only part of the genetic diversity reached their current locations, i.e. the population has gone through a genetic bottleneck, deserves less credit, as the geographic range of the specimens studied was wide (from Bulgaria to Scandinavia).

For a sustainable identification of animals, Hebert et al. (2003) proposed a system that employs DNA sequences as taxon “barcodes”. They suggested that the mitochondrial COI gene could serve as the core of a global biodiversity identification system. Our results tend to support their proposal, although with reservations. As two *Timandra* specimens were placed into the “wrong” clade, we suggest that complete lineage sorting and lack of hybridization are necessary preconditions to be fulfilled before the use of “barcoding” is justified.

**CONCLUSIONS**

The phylogenetic structure, colonization history, phenology and current distribution of *T. griseata* and *T. comae* seems to be an example of the evanescence of geographic barriers between populations, which has resulted in hybridization, albeit with low frequency. As the populations nevertheless appear to retain their integrity, separation of *T. griseata* and *T. comae* into two separate species is warranted.
REFERENCES

AARVIK L., BERGGREN K. & HANSEN L.O. 2000: Catalogus Lepidopterorum Norvegiae. LepArb, Zool. Mus., NISK, Oslo, 192 pp.

BANDELT H.-J., FORSTER P. & RÖHL A. 1999: Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16: 37–48.

CATERINO M.S. & SPERLING F.A.H. 1999: Papilio phylogeny based on mitochondrial cytochrome oxidase I and II genes. Mol. Phylog. Evol. 11: 122–137.

CATERINO M.S., CHO S. & SPERLING F.A.H. 2000: The current state of insect molecular systematics: A thriving tower of Babel. Annu. Rev. Entomol. 45: 1–54.

FUNK D.J. & OMLAND K.E. 2003: Species-level paraphyly and polyphyley: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34: 397–423.

GORDON D., ARAIAN C. & GREEN P. 1998: Consed: a graphical tool for sequence finishing. Gen. Res. 8: 195–202.

HAUSMANN A. 1997: The geometrid moths of various entomological collections in Israel (Lepidoptera, Geometridae). Entomofauna 18: 1–20.

HAUSMANN A. 1999: The Geometrid Moths of Europe. Vol. 2. Sterrhinae. Apollo Books, Stenstrup, 600 pp.

HALL T.A. 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41: 95–98.

HARRISON R.G. 1998: Linking evolutionary pattern and process: the relevance of species concepts for the study of speciation. In Howard D.J. & Berlocher S.H. (eds): Evolutionary Pattern and Process: The Relevance of Species Concepts for the Study of Speciation. Oxford University Press, Oxford, pp. 19–31.

HEBERT P.D.N., CYWINSKA A., BALL S.L. & DEWAARD J.R. 2003: Biological identifications through DNA barcodes. Proc. R. Soc. Lond. (B) (Biol. Sci.) 270: 313–321.

HÖLZEN L. (ed.) 2000: Suomen suurperhosatlas. Finlands storfjärilaras. [Atlas of Finnish Macrolepidoptera.] Suomen Perhostutkijain Seura, Helsinki, 328 pp.

ICZN 1985: International Code of Zoological Nomenclature. 3rd ed. The International Trust for Zoological Nomenclature, c/o The Natural History Museum, London, 338 pp.

ICZN 1999: International Code of Zoological Nomenclature. 4th ed. The International Trust for Zoological Nomenclature, c/o The Natural History Museum, London, 306 pp.

INOUE H., SUGI S., KUROKO H., MORIUTI S. & KAWABE A. 1982: Moths of Japan. Vol. 2. Plates and Synonymic Catalogue. Kodansha, Tokyo, 396 pp.

KAILA L. & ALBRECHT A. 1994: The classification of the Timandra griseata group (Lepidoptera: Geometridae, Sterrhinae). Entomol. Scand. 25: 461–479.

KAILA L. & ALBRECHT A. 1995: The geometrid genus Timandra (Lepidoptera, Geometridae) contains two species in Finland. Baptia 20: 149–156 [in Finnish, English abstr.]

KAILA L., PEDMANSON R. & TAMMARU T. 1999: Two species of Timandra (Lepidoptera, Geometridae) occur in Estonia. Lepid. Inform. 11: 7–8 [in Estonian, English abstr.]

KAISILA J. 1954: Über das vorkommen zweier Generationen bei den finnischen Grossschmetterlingen im allgemein und besonders im Sommer 1953. Ann. Entomol. Fenn. 20: 20–40.

KULLBERG J., ALBRECHT A., KAILA L. & VARIS V. 2002: Checklist of Finnish Lepidoptera – Suomen perhosten luettelo. Sahlbergia 6(2): 45–190.

KUMAR S., TAMURA K., JAKOBSEN I.B. & NEI M. 2001: MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17: 1244–1245.

LARSEN K. 1995: Timandra griseata Petersen, 1902 – new for Fauna of Denmark. Entomol. Meddr. 63: 53–55 [in Danish].

MAYR E. 1963: Animal Species and Evolution. Belknap Press, Cambridge, MA, 811 pp.

MÜLLER M.A., HAUSMANN A. & TRUSCH R. 2001: The phylogenetic relationships in Geometrid moths. An approach using mitochondrial DNA (mtDNA) sequences. In Hausmann A. & Trusch R. (eds): Proceedings of the FORUM HERBULOT 2001 Neotropical Geometridae: Approaches to a Modern Concept of the Geometrid System on Genus and Tribe Level (8.3.–9.3.2001). Spixiana 24: 201–202.

MÜLLER B. 1996: Geometridae. In Karsholt O. & Razowski J. (eds): The Lepidoptera of Europe. A Distributional Checklist. Apollo Books, Stenstrup, pp. 218–249.

PAGE R.D. 1996: TreeView: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12: 357–358.

PALMIQVIST G. 1997: Remarkable records of Macrolepidoptera in Sweden 1996. Entomol. Tidsskr. 118: 11–27 [in Swedish, English abstr.]

PAMILLO P. & NEI M. 1988: Relationships between gene trees and species trees. Mol. Biol. Evol. 5: 568–583.

PITTAWAY A.R. 1993: The Hawkmoths of the Western Palaearctic. Harley Books, Colchester, 240 pp.

POSADA D. & CRANDALL K.A. 1998: MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817–818.

PROUT L.B. 1935–38: Die Amerikanischen Spanner. In Seitz A. (ed.): Die Gross-Schmetterlinge der Erde. Vol. 8: 57–59.

RONQUIST F. & HUELSENBECK J.P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

RONQUIST F. & HUELSENBECK J.P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

SAYKONOV N. & ŠULCS I. 2004: New and rare Lepidoptera for the Latvian fauna. Report No 15. Baptia 29: 52–58.

SCHMIDT A. 1931: Eine neue Timandra-Forn aus Spanien. Int. Entomol. Z. 25: 57–59.

SCORLE M. (ed.) 1999: Geometricoths of the World. I-II. CSIRO & Apollo Books, London, 1016 + 129 pp.

SHVONEN P. 2001: Evorted vesicale of the Timandra griseata group: methodology and differential features (Geometridae: Sterrhinae). Nota Lepid. 24: 57–63.

SWOFFORD D.L. 1998: PAUP*: Phylogenetic Analysis Using Parsimony (*And Other Methods), Version 4.0610. Sinauer Associates, Massachusetts.
THOMPSON J.D., HIGGS D.G. & GIBSON T.J. 1994: ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22: 4673–4680.

TRUSCH R., MILLER M.A., HILLE A. & HAUSMANN A. 2002: Unravelling the Gordian knot: molecular approach to a new and better understanding of sibling species complexes in geometrid moths. *Proceedings of the 13 European Congress of Lepidopterology*. Zoological Museum, Copenhagen, pp. 59–60.

VIIADELP J. 1996: *Checklist of the Geometridae (Lepidoptera) of the Former U.S.S.R*. Apollo Books, Stenstrup, 109 pp.

VIIADELP J. 2000: *Parnassius mnemosyne Linnaeus, 1758 in Estonia*. Abiks loodusevaatlejale No. 98. Tallinn-Tartu, 40 pp. (in Estonian, English abstr.).

WAHLBERG N., OLIVEIRA R. & SCOTT J.A. 2003: Phylogenetic relationships of Phyciodes butterfly species (Lepidoptera: Nymphalidae): complex mtDNA variation and species delimitations. *Syst. Entomol.* 28: 257–273.

WIENS J.J. & PENKROT T.A. 2002: Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (Sceloporus). *Syst. Biol.* 51: 69–91.

Received December 31, 2004; revised and accepted March 11, 2005
Twenty-nine species belonging to the subfamily Sterrhinae (Geometridae) are recorded from Central and North-west Turkmenistan. The description of a new species Idaea laszloi sp. nov. Hausmann is given. \cite{Hausmann2003}. Phylogenetic evaluation of the taxonomic status of Timandra griseata and T. comae (Lepidoptera: Geometridae: Sterrhinae). European Journal of Entomology 102(4): 607-615, 2005. New records and critical notes on Geometridae of Emilia, Romagna and Tuscany Part I Insecta Lepidoptera, Geometridae Ennominae, Oenochrominae, Geometrinae, Sterrhinae Nuove segnalazioni e note critiche sui geometridi di Emilia, Romagna e Toscana I parte Insecta Lepidoptera, Geometridae Ennominae, Oenochrominae, Geometrinae, Sterrhinae. The blood-vein, (Timandra comae) is a moth of the family Geometridae. YouTube Encyclopedic. 1/3. Å–unap, Erki, Viidalepp Jaan and Saarma, Urmas \cite{Unap2005}. Phylogenetic evaluation of the taxonomic status of Timandra griseata and T. comae (Lepidoptera: Geometridae: Sterrhinae). Eur. J. Entomol., 102:607-615. online. Skinner, Bernard \cite{Skinner1984} Colour Identification Guide to Moths of the British Isles 1984. External links. Wikimedia Commons has media related to Blood-vein. \cite{Wikimedia2016} Taxonomic Notes on the Genus Orthobrachia Warren (Lepidoptera, Geometridae), with Description of A New Species from China and Thailand. A O. tenebrosa Yazaki, 1992, male from Nepal, paratype; B O. owadai Yazaki, 1992, female from Nepal, paratype; C O. simpliciata Yazaki, 2002 C male from China, paratype; E O. maoershanensis Huang, Xin & Wang, 2003, male from Guangxi Province in China, holotype; G–H O. hirowatarii Huang, Su & Stüning, sp. n. G male from Sichuan Province in China, holotype H female from Sichuan. Å In contrast, the relatively conserved morphology of the posterior part of the body across the genus suggests that fossoriality mostly involves the anterior part.