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Pleistocene occurrence of recently discovered cryptic vermetid species from the Mediterranean

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ABSTRACT - Significant genetic differences between populations previously assigned to Dendropoma petraeum *(Monterosato, 1884) from diverse Mediterranean localities have been recently highlighted. Molecular analyses disclosed a complex of at least four cryptic species with highly diverging sequences coupled to a low degree of morphological differentiation or stasis in the adult stages. Differences were found in the early development of intracapsular larval shells. In the present paper the use of morphology and size of intracapsular larval shells preserved in the fossil record is proposed in order to trace genetic divergence in the geological past. The application of this research method in the study of Upper Pleistocene representatives of* Dendropoma *from Eastern Sicily, allowed documentation of the persistence of the expected clade for the area, at least since the Tyrrhenian Stage. This time interval, of the order of one hundred thousand years, is very short, when compared to the 4.5-17 Myr period indicated as a possible time frame for divergence utilizing the molecular clock. It is suggested that examination of protoconchs of* Dendropoma *specimens from selected older outcrops within the Mediterranean area could in this case provide more precise information with respect to the extremely broad interval obtained utilizing the molecular clock.*

RIASSUNTO - [Evidenze pleistoceniche di una specie criptica di vermetide recentemente scoperta dal Mediterraneo] *- Significative differenze genetiche sono state recentemente messe in evidenza fra popolazioni precedentemente attribuite a* Dendropoma petraeum *(Monterosato, 1884) da località diverse del Mediterraneo. Recenti analisi molecolari hanno infatti dimostrato come questo taxon corrisponda a un complesso di quattro diverse specie criptiche, caratterizzate da sequenze altamente divergenti associate a una differenziazione morfologica dell'adulto lieve o assente. Alle differenze genetiche e nello sviluppo intracapsulare si associano variazioni nella taglia e morfologia delle protoconche, il cui uso viene qui suggerito per tracciare la divergenza genetica delle diverse popolazioni nel passato geologico. Le protoconche, infatti, conservate nella documentazione fossile possono essere facilmente esaminate. L'applicazione di questo metodo a esemplari di* Dendropoma *del Pleistocene Superiore della Sicilia orientale documenta la persistenza del clade atteso per l'area almeno dal Tirreniano, un intervallo di tempo relativamente breve se confrontato con i 4,5-17 milioni d'anni indicati come possibile ambito temporale per la divergenza applicando l'orologio molecolare. Si rimarca come l'esame di conchiglie intracapsulari di esemplari selezionati di* Dendropoma *provenienti da affioramenti più antichi dell'area mediterranea potrebbe in questo caso fornire informazioni più precise rispetto all'intervallo estremamente ampio dell'orologio molecolare.*

INTRODUCTION

The vermetid gastropod *Dendropoma* is comprised of species secreting tubular shells that are irregularly coiled and cemented to the substratum and to each other in gregarious species. Settlement of offsprings on and near the parental individuals and gregariousness are favoured by internal brooding and a larval lecitotrophic development that is entirely intracapsular (e.g., Calvo et al., 1998). Because of these behaviours *Dendropoma*, often associated with *Vermetus* species and calcareous algae, is able to build important bioconstructions in temperate to subtropical areas, mostly in the Atlantic Ocean (Caribbean region, Brazil and West Africa) as well as in the Mediterranean, plus isolated records in the Eastern Pacific Ocean (Safriel, 1974; Chemello, 2009; Spotorno et al., 2012). Bioconstructions exhibit different morphologies but invariably grow in a narrow belt near the sea level. In the Mediterranean, *Dendropoma* buildups develop in the warmest sectors, along particularly exposed rocky shores, in the lower midlittoral fringe.

Consequently, these *Dendropoma* buildups are considered as extremely useful palaeoecological indicators and their recognition has been exploited to infer past sea surface temperature (Antonioli et al., 1999; Silenzi et al., 2004; Montagna et al., 2008) and to reconstruct palaeo-shorelines (Laborel & Laborel-Deguen, 1994; Schiaparelli et al., 2006). Observation of the elevation of *Dendropoma* buildups above present-day sea level coupled with shell radiocarbon dating and information about sea level eustatic changes, contributed to the reconstruction of the tectonic history of several areas of the Mediterranean (see, among others: Pirazzoli et al., 1996; Antonioli et al., 1999; Morhange et al., 2006).

Dendropoma buildups reach considerable development when sea level is stable or rises slowly. Consequently, the present-day structures probably started to develop not earlier than about 6,000 years ago (Silenzi et al., 2004), although it was demonstrated that such bioconstructions are locally not older than a few centuries (Antonioli et al., 1999).

A single species of *Dendropoma* was recognised until short time ago (Calvo et al., 2009) and morphological

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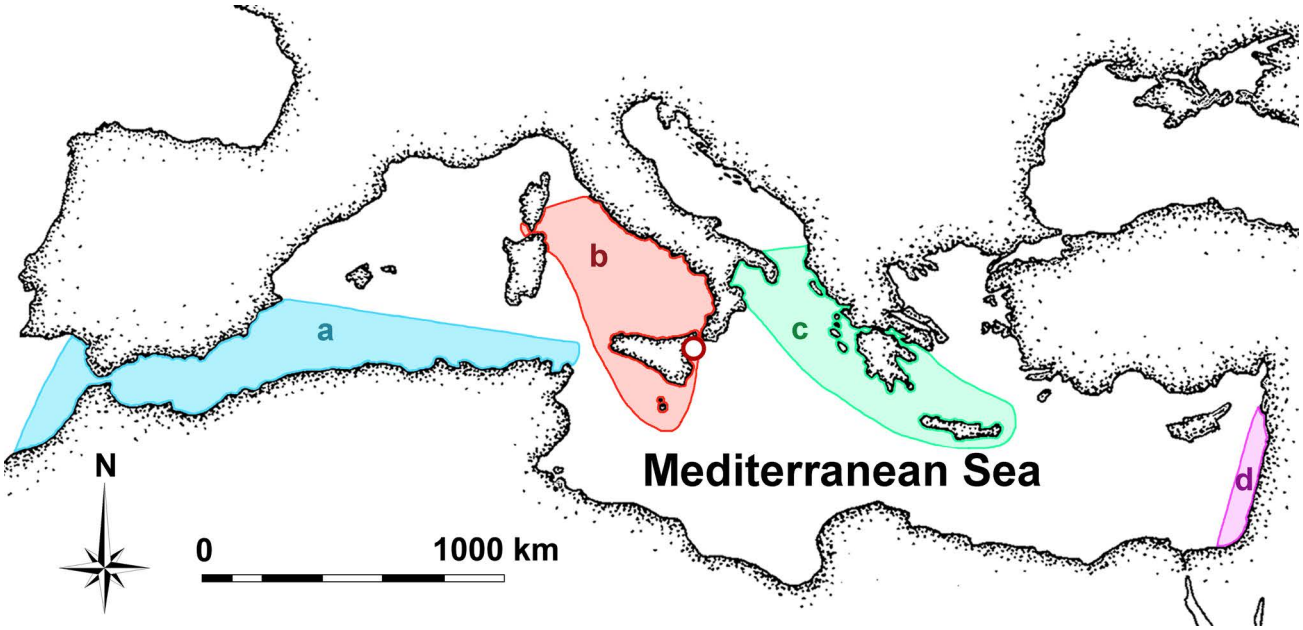

Fig. 1 - Location of the studied Pleistocene outcrop plotted against the distribution of the four Mediterranean present-day *Dendropoma* species. Asterisk indicates the Taormina cave preserving fossil *Dendropoma* specimens. Letters a-d indicate the geographical ranges of species first recognised as clades by Calvo et al. (2009), redrawn from their fig. 2. a) "Western" clade; b) "Tyrrhenian-Sicilian" clade; c) "Ionian-Aegean" clade; d) "Levantine" clade.

variation between specimens belonging to populations from different areas was considered intraspecific. Phylogenetic analyses on specimens collected in several localities of the Mediterranean Sea and the near Atlantic Ocean lead Calvo et al. (2009, 2015) to identify four cryptic species restricted to specific areas. These correspond to the series of "Western", "Tyrrhenian-Sicilian", "Ionian-Aegean" and "Levantine" clades (Fig. 1). Following Calvo et al. (2009) the "Tyrrhenian-Sicilian" clade including the type locality, corresponds to *D. petraeum* (Monterosato, 1884), now *D. cristatum* (Biondi, 1859), and the four Mediterranean species can be distinguished based on their reproduction behaviour through intracapsular brooding of larvae, the number of capsulae contemporaneously developing within a female and the total number of eggs brooded per capsula, as well as for the sizes of eggs and egg capsulae.

The morphology of the intracapsular larval shells, although figured, was not considered diagnostic to distinguish the four Mediterranean species of *Dendropoma* by Calvo et al. (2009); it was only introduced as a difference between *D. cristatum* and a new species in Templado et al. (2016), a paper published after the submission of this contribution. Vermetid intracapsular shells can be easily recognised because of their ornamentation and obvious rims delimiting them from the subsequent teleoconch. Unlike in other gastropods, these protochoncs are protected from abrasion owing to the special morphology and the encrusting life habit of adult vermetids, and can be disclosed inside shells.

The aim of this paper is to look for the possible preservation of *Dendropoma* protoconchs in the fossil record in order to provide information to the understanding of the history of the divergence of this group of species as suggested by Calvo et al. (2009, 2015).

MATERIAL AND METHODS

The examined material comes from a cave carved in Mesozoic carbonate rocks, and located in the promontory of Taormina in NE Sicily (Bonfiglio, 1981), near the homonym town (Fig. 1). The cave, nearly 23 m long and 8 m wide, has a large opening and is presently located at about 130 m a.s.l. Biogenic vermetid crusts, a few centimetres thick, are present along the cave walls. They formed when the cave was semi-submerged during the Upper Pleistocene, but before the Tyrrhenian Stage, according to the position of the cave above the Tyrrhenian terrace (Bonfiglio et al., 2015).

Small samples were collected and examined under a stereomicroscope for species identification. Thanks to the local dissolution, some tubes were accurately separated in order to observe the protoconchs. Selected uncoated protoconchs were observed and photographed using a Tescan Vega 2 LMU, Low Vacuum Scanning Electron Microscope, with backscattered produced images.

Material is housed in the collections of the Palaeontological Section of the Museo di Scienze della Terra of the University of Catania, under the code number PMC R-S.I.Ps.M.1a.

RESULTS

Crusts sampled inside the cave revealed to be entirely formed by vermetids belonging to *Dendropoma cristatum* (Figs 2-3). Protoconchs were discovered still in place with tubes of adult shells (Fig. 4a) or isolated within the collected samples (Fig. 4b1). They slightly etch the substratum and consequently can be easily detached, probably also because of the incipient dissolution

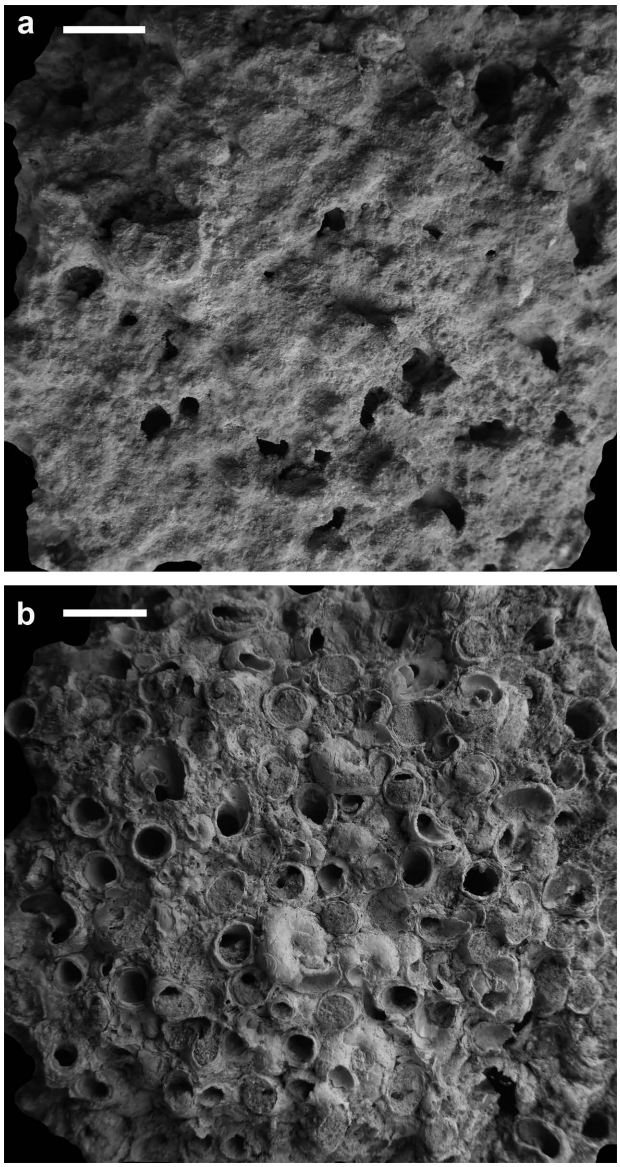

Fig. 2 - Appearance of the Upper Pleistocene concretions produced by specimens belonging to *Dendropoma cristatum* (Biondi, 1859). a) External surface of the bioconcretion with barely visible specimens. Scale bar corresponds to 1cm. b) Cut surface of the same hand sample showing the piling up of gastropod shells, partly filled by sediment. Scale bar corresponds to 1 cm.

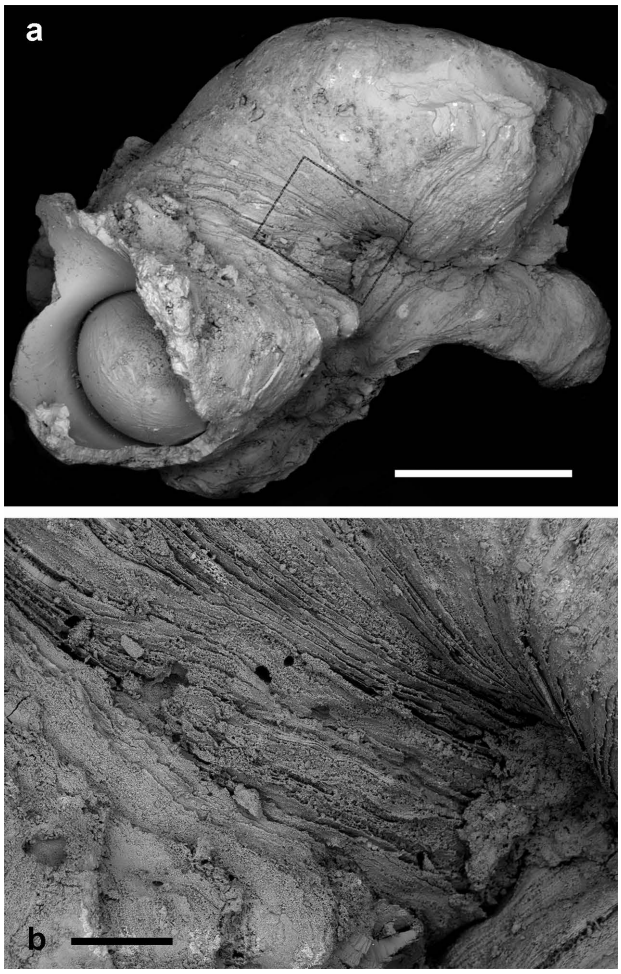

Fig. 3 - Shell features of *Dendropoma cristatum* (Biondi, 1859) from the Upper Pleistocene concretions of the Taormina cave. a) Fragment of a shell showing the typical ornamentation. An internal hemispherical septum is visible. Scale bar corresponds to 5 mm. b) Detail of the shell surface from the rectangle indicated in Fig. 3a. Scale bar corresponds to 500 µm.

enhancing separation of skeletons constituting the early vermetid framework.

The protoconch, having a mean diameter of 680.0 µm (Tab. 1) includes 1.3 whorls and consists of two portions differently ornamented. The former one-whorl portion appears smooth at low magnification, but shows a gently wrinkled surface when examined under SEM (Fig. 4b2-3). The latter portion shows a well evident axial ornamentation consisting of a dozen of gently undulated ribs, that are about 50 µm spaced, except for the last ones that become slightly more closely spaced (Figs 4a2 and 4b1, 3-4). Their inspection (Fig. 4b4) suggests they result from folding of the shell layer.

The protoconch is clearly delimited from the subsequent teleoconch portion by a rim having the same undulating pattern of ribs. The first portion of the teleoconch is irregularly tubular, the diameter is larger than the protoconch and rapidly increases in size; the shell shows a variable thickness and an undulating surface sculptured by faint, largely apart and irregularly shaped transversal ornamentations (Fig. 3b) that correspond to growth increments and possible subsequent tube reorientations (see Schiaparelli & Cattaneo-Vietti, 1999). Internal lamellae are present at intervals within the tubular shell cavity (Fig. 3a).

DISCUSSION AND CONCLUSION

The observed protoconchs from the Taormina cave are fully comparable to the intracapsular larval shells of *D. petraeum* 'Central clade', figured by Calvo et al. (2009: fig. 4B) and Milazzo et al. (2014: fig. 3C, E, G, I) and of *D. cristatum* by Templado et al. (2016: fig. 3B). All of them share the same total number of whorls, the type of ornamentation and the relative proportions of the differently ornamented portions. Furthermore, sizes are

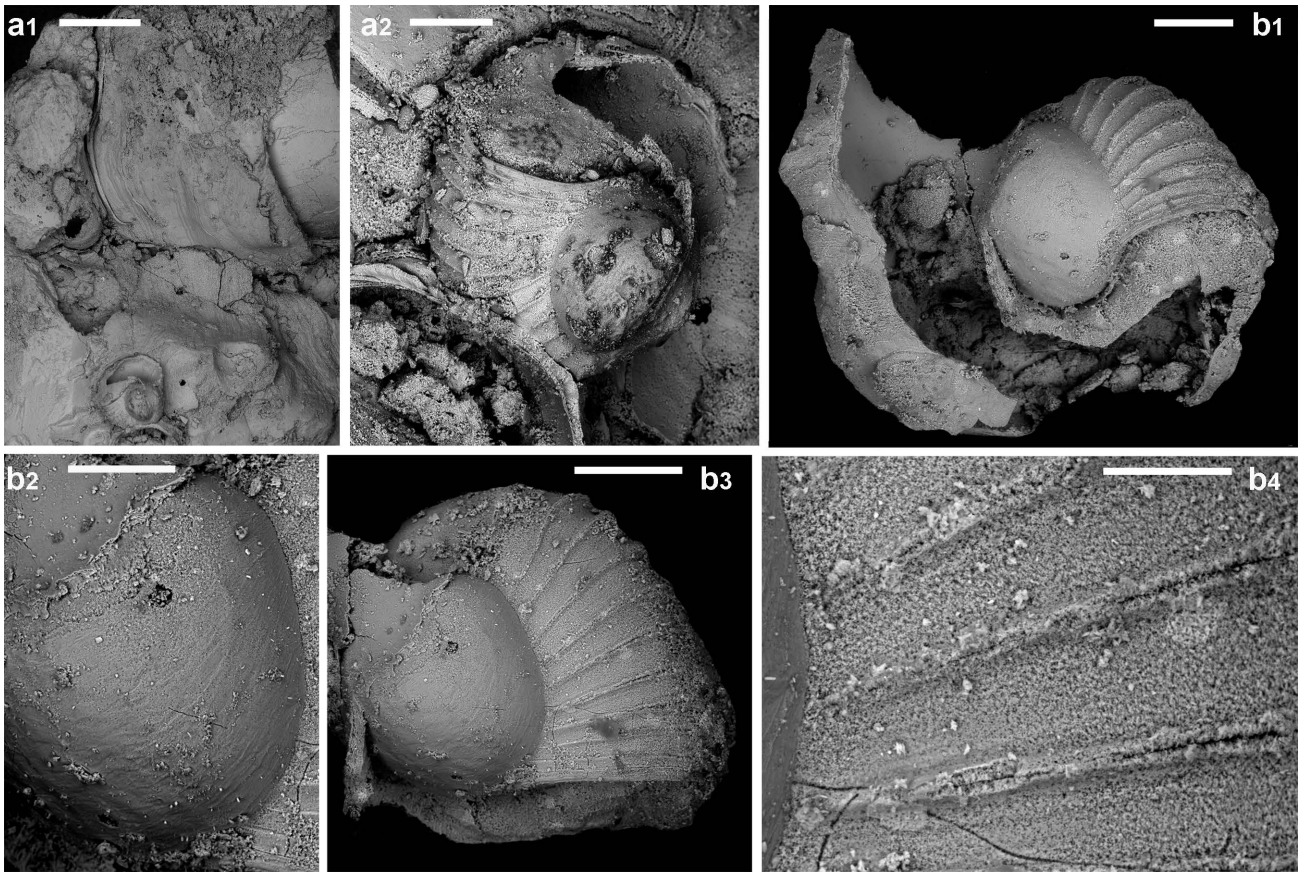

Fig. 4 - Features of the protoconch of *Dendropoma cristatum* (Biondi, 1859) from the Upper Pleistocene concretions of the Taormina cave. a1) Concretion fragment consisting of adult shell portions on which a juvenile specimen (lower centre) is preserved. Scale bar corresponds to 1mm. a2) Close-up of the protoconch shown in Fig. 4a1. Scale bar corresponds to 200 µm. b1) An isolated juvenile shell fragment including the protoconch. Scale bar corresponds to 200 µm. b2) Close-up of the initial portion of the protoconch shown in Fig. 4b3. Note the faint drapery-shaped ornamentation. Scale bar corresponds to 100 µm. b3) Same protoconch of the specimen illustrated in Fig. 4b1 with the typical ornamentation of *Dendropoma cristatum* (Biondi, 1859). Scale bar corresponds to 200 µm. b4) Close-up of the final portion of the protoconch shown in Fig. 4b3. Note the folded nature of the axial costae. Scale bar corresponds to 50 µm.

strikingly comparable, as Upper Pleistocene specimens have total diameters largely overlapping with the 690 µm and 720 µm diameters estimated from the figures in Calvo et al. (2009, 2015). The folded nature in the construction of axial costae (not described by Calvo et al., 2009, nor by Templado et al., 2016) is confirmed by the comparison of the presumably partly dissolved protoconch in Fig. 4b4 with the present-day intracapsular larval shells, exposed to low aragonite saturation in acidification experiments (fig. 3L of Milazzo et al., 2014). In both instances costae show the double layered structure resulting from folding, and highlighted by the dissolution of the small prismatic crystals forming the rib apical portions.

Interestingly, and as expected, Upper Pleistocene protoconchs from the Taormina cave differ strongly from the intracapsular larval shells of the Western (now *D. lebeche* Templado Richter & Calvo, 2016) and Ionian-Aegean clades figured by Calvo et al. (2009, fig. 4A and C), which are characterised by larger and smaller sizes, respectively, and by decidedly different ornamentations lacking axial elevated arched costae, and only including more or less evident growth lines.

All the above evidences suggest that protoconchs can be confidently used to distinguish vermetid species having similar adult specimen features. Indeed, protoconch characters not used by Calvo et al. (2009) were later considered among the diagnostic features to useful distinguish *Dendropoma* species by Calvo et al. (2015).

Specimens from the Taormina cave identified as belonging to *D. cristatum* document the occurrence of this species in the Upper Pleistocene, in an area comprised within its present-day geographical distribution. This time interval is very small in comparison to the presumed age (4.5 to 17 Myr) for the divergence event between

Specimens	1	2	3	4	5	6	7	8	9	10	11	12	min	max	mean	sd
Protoconch diameter	697	675	670	682	671	673	672	666	670	703	712	669	666	712	680.0	15.3

Tab. 1 - Measurements (in µm) of 12 protoconchs of *Dendropoma cristatum* (Biondi, 1859) from the Upper Pleistocene concretions of the Taormina cave. The minimum (min), maximum (max) and mean measurements are reported together with the standard deviation (sd).

the four Mediterranean species, as postulated trough the application of the Bayesian clock (Calvo et al., 2009) and, subsequently, considered as the times corresponding to two possible options of slow and fast divergence rates suggested by Calvo et al. (2015). This case elucidates that the Bayesian method is largely biased by the assumption of standard clock rate of divergence, therefore it needs to be rooted through palaeontological documentation. *Dendropoma* protoconchs with their preservable and easily detectable diagnostic characters, supply a decidedly more reliable distribution datum. On this side, the main effort will be to discover Lower-Middle Pleistocene to Pliocene (or even more ancient, if any) *Dendropoma* buildups and specimens from different localities in the Mediterranean area (and outside) to trace back confidently the persistence of the present-day species in the geological past. These findings will provide new information to better delineate the evolution of *Dendropoma* lineages and to question or substantiate inferences about the divergence time inferred using the molecular clock.

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