Molecular clocks indicate turnover and diversification of modern coleoid cephalopods during the Mesozoic Marine Revolution

Tanner, Alastair R.; Fuchs, Dirk; Winkelmann, Inger Eleanor Hall; Gilbert, Tom; Pankey, M. Sabrina; Oliveira Ribeiro, Ângela Maria; Kocot, Kevin M.; Halanych, Kenneth M.; Oakley, Todd H.; Rodrigues da Fonseca, Rute Andreia; Pisani, Davide; Vinther, Jakob

Published in:
Proceedings of the Royal Society B: Biological Sciences

DOI:
10.1098/rspb.2016.2818

Publication date:
2017

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY

Citation for published version (APA):
Tanner, A. R., Fuchs, D., Winkelmann, I. E. H., Gilbert, T., Pankey, M. S., Oliveira Ribeiro, Â. M., Kocot, K. M., Halanych, K. M., Oakley, T. H., Rodrigues da Fonseca, R. A., Pisani, D., & Vinther, J. (2017). Molecular clocks indicate turnover and diversification of modern coleoid cephalopods during the Mesozoic Marine Revolution. Proceedings of the Royal Society B: Biological Sciences, 284(1850), [20162818].
https://doi.org/10.1098/rspb.2016.2818
Molecular clocks indicate turnover and diversification of modern coleoid cephalopods during the Mesozoic Marine Revolution

Alastair R. Tanner1, Dirk Fuchs3, Inger E. Winkelmann4,
M. Thomas P. Gilbert4,5,6, M. Sabrina Pankey7, Ângela M. Ribeiro4,
Kevin M. Kocot9, Kenneth M. Halanych10, Todd H. Oakley11,
Rute R. da Fonseca4,8, Davide Pisani1,2 and Jakob Vinther1,2

1 School of Biological Sciences, and 2 School of Earth Sciences, University of Bristol, Life Sciences Building, 24 Tyndall Avenue, Bristol BS8 1TQ, UK
3 Earth and Planetary System Science, Department of Natural History Sciences, Hokkaido University, Sapporo, Japan
4 Natural History Museum of Denmark, Øster Voldgade 5-7, 1350 Copenhagen, Denmark
5 Trace and Environmental DNA Laboratory, Department of Environment and Agriculture, Curtin University, Perth, Western Australia, Australia
6 NTNU University Museum, Norwegian University of Science and Technology, Trondheim, Norway
7 Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, NH 03824, USA
8 Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen N, Denmark
9 Department of Biological Sciences, University of Alabama, Box 870344, Tuscaloosa, AL 35487, USA
10 Department of Biological Sciences, Auburn University, Auburn, AL 38633, USA

ART, 0000-0001-8045-2856; DP, 0000-0003-0949-6682; JV, 0000-0002-3584-9616

1. Introduction

Octopus, cuttlefish and squid showcase advanced intelligence, a wide range of body sizes, sophisticated camouflage and mimicry, unique jet-locomotion and ingenious decoy countermeasures in the ink sac [1–3]. Charismatic in these ways, and owing to their importance as fishing stocks, cephalopods have...
garnered great interest from ecologists and evolutionary biologists. However, cephalopod evolutionary relationships and divergence times have remained unclear, in part, owing to uncertainties in their fossil record. The past 540 Ma of cephalopod evolution can be viewed as having three ecologically distinct phases. Originally shelled, sea-floor-dwelling molluscs, cephalopods are descended from superficially limpet-like ancestors in the Cambrian [4,5]. The protective shell later became adapted as a chambered buoyancy organ [6], giving rise to free-swimming forms by the latest Cambrian, while cirrate forms and stem octobrachians are recorded in the Jurassic [13]; these are known to preserve portions of the shell (phragmocone and rostrum) has a high potential for preservation, but as the phragmocone became internalized, reduced, and in many cases lost entirely, so too was a clear narrative through fossils. Soft tissue fossilization is rare, but cirrate and incirrate octopods are known from the Late Cretaceous (Cenomanian) Häkel and Hädjoula Lagerstätte, while cirrate forms and stem octobrachians are recorded in the Jurassic [13]: these are known to preserve the unmineralized gladius and soft tissues. Stem group decabrachians, such as belemnites and other belemnoids are known, preserving their phragmocones and, occasionally, soft tissues [14,15]. By contrast, the extant octopuses, cuttlefish, and squid are characterized by shell reduction and loss [16], and are prone to major taphonomic biases in tissue preservation [14]. Consequently, clarifying evolution of coleoids from the Mid-Palaeozoic to the present must, therefore, rely on alternative palaeobiological approaches, such as the estimation of molecular divergence times.

The first molecular divergence times of cephalopod evolution recovered very ancient divergences for the coleoids [17],
Figure 2. Phylogeny of 26 cephalopod species, plus outgroups (further details in figure 1); 180 genes, concatenated as 36 156 aligned amino acid positions with 26% missing data, modelled under CAT + GTR + I. Numbers at nodes denote Bayesian posterior probability/bootstrap support as returned by RAxML under the LG [33] substitution mode. Dotted branches at base of phylogeny are shortened for clarity, and outgroups (26 gastropods and bivalves, one scaphopod, four annelids) are collapsed for clarity (figure 1). Scale bar is expected substitutions per site.

suggesting extensive gaps in the fossil record. However, these studies used controversial calibrations from the Late Palaeozoic, such as *Shimanskya* [18] and *Pohlsepia* [19], for which the assignment to the coleoid crown group is dubious [20]. Subsequent studies attempted to estimate cephalopod divergences using calibrations from outgroups, such as bivalves and gastropods and recovered much younger divergence estimates, that were surprisingly congruent, irrespective of differences both in methodology and gene sampling [20,21]. These independent studies recovered a divergence between the nautiloids and the coleoids around the Silurian–Devonian boundary, or the earliest Devonian (approx. 415 Ma), which is congruent with unequivocal evidence for fossil stem group coleoids (ammonoids and bactritids) [22,23] and stem group nautiloids [24] in the Early Devonian. Cephalopod beaks also appear in the fossil record in the Devonian [25]. These observations suggest that the fossil record documents the origin of the crown group and that the concomitant evolution of the beak [20] coincides with a dramatic shift in predator–prey dynamics, termed the Devonian Nekton Revolution [26]. The jawed vertebrates radiated at this time, incident with a global shift in predatory style towards increased high-metabolism predation and durophagy [27]. The coincidence of jawed vertebrates and beaked cephalopods radiating at the Silurian–Devonian boundary may thus be interpreted as a response to the changes in the predator–prey landscape.

To explore the tempo and mode of coleoid evolution, we assembled a dataset of 180 nuclear genes of consistent rate of molecular evolution, representing crown diversity across Coleoidea. Phylogenetic and molecular divergence time analyses were carried out in a Bayesian framework, applying a molecular evolution model accommodating rate and compositional heterogeneity.

2. Experimental procedures

For full details of experimental procedures, see the electronic supplementary material. We compiled a supermatrix with data from 56 species (electronic supplementary material, table S2) for 180 genes. Phylogeny was inferred from this superalignment using the software package *PhyloBayes* MPI v. 1.5a [28] under CAT + GTR + I. The maximum-likelihood software RAxML MPI v. 8.1.15 [29] was applied to the same dataset as used in Bayesian inference, applying LG + I + G.

*PhyloBayes* 3.3f was used to infer molecular divergence times under the CIR [30] clock model, soft-bounds of 0.05 and a Yule-process birth–death model, with topology fixed to that inferred by *PhyloBayes* MPI v. 1.5a. A prior was applied to the root of 565 ± 10 Ma, representing the root of lophotrochozoa. Eleven fossil calibration points were applied to the analysis, as shown in table (electronic supplementary material, table S1).

3. Results

Our phylogenetic results confirm *Nautilus* as sister group to coleoids [20,31]. In turn, coleoids comprise two monophyletic groups: Octobrachia (Vampire squid, dumbo octopuses and...
incirrate octopuses) and Decabrachia (cuttlefish and squid, including Spirula), in agreement with morphology and previous molecular studies [16,17,32] (figure 1). The vampire squid Vampyroteuthis and the cirroctopod Grimpoteuthis represent cirrate octopuses, branching deep as successive sister groups to the incirrate octopuses (figure 1). Within Decabrachia, we recover a monophyletic Myopsida assemblage, along with support for Teuthoidea with the inclusion of Spirula, similar to previous studies [16,20]. However, the relationships between the orders comprising the Sepioidea (Sepiida, Idiosepiidae, Sepiolidae) are recovered as paraphyletic. Oegopsid monophyly is supported, with Spirula sister to this clade, in agreement with previous studies [16], but the posterior probability values for many decabrachian basal nodes are generally lower than in other parts of the phylogeny. Sepioid and myopsid relationships have proved difficult to resolve [16], and further phylogenetic work remains to clarify these.

Molecular divergence times were estimated, from the same matrix used for phylogenetic inference, applying an autocorrelated relaxed clock model (CIR process, figures 2 and 3; electronic supplementary material for further details and additional analyses). Alternative treatments, model applications and comparison of the joint priors induced by our calibrations and models and the posterior divergence times supported the data as informative, and resulted in consistency in divergence time inference (figure 3; electronic supplementary material, table S3 and figure S3). Notably, our molecular divergence times are highly congruent with previous molecular divergence estimates [20,34] that used comparable calibration schemes. These studies, however, had insufficient taxonomic spread and sample required for more comprehensive investigation of the evolutionary tempo of coleoids. Furthermore, our wide sample represents crown diversity.

The oldest unequivocal crown group coleoids appear in the latest Triassic, with belemnites representing stem group decabrachians, and phragmoteuthidids (Early Triassic or latest Permian) proposed to represent stem group Octobrachia [35]. Our divergence times suggest that the coleoid crown diverged in the Late Carboniferous or Permian. Fossil consilience is shown by stem group vampire squid (loligosepiids) fossils of the earliest Jurassic (approx. 195 Ma) [13,36]. Octopus-like forms that are lacking the mantle fins and with reduced gladius appear in the latest Cretaceous (Cenomanian, 94–100 Ma) Lagerstätte of Häkel and Hâdjoula, Lebanon [37].
4. Discussion

Our molecular divergence estimates show that the coleoid fossil record [13,39] belies not only an earlier origin for key cephalopod groups, but also significant differences in their rate of diversification. Together with the molecular clock estimates for coleoids that are lacking a fossil record, it is possible to investigate events that shaped the diversity of the group. Decabrachi ans diversify rapidly in the middle Mesozoic (Jurassic), while incirrate octopuses arose in the Cretaceous. Since this time documents an escalation—the evolution of novel predation strategies—it prompts a consideration of what anatomical changes took place in coleoids, particularly decabrachi ans, at this time.

The iconic shell has had a shifting functional role through cephalopod evolution, and is informative as to lifestyle and ecology. Subsequent to ancestral internalization of the phragmocone through the Carboniferous and Devonian, the decabrachian and octobrachian lineages independently evolved towards shell reduction [13,16], allowing enhanced manoeuvrability and speed [15]. These groups would have been in ecological competition with belemnites: stem group decabrachi ans [39,40] with an elaborate internal shell, diversifying in the Mid-Jurassic [41]. Our analysis suggests that in the Late Jurassic and at the onset of the Cretaceous, belemnites became marginalized and replaced by modern groups of decabrachi ans and finned octobrachi ans (figure 2) [13]. By retaining an elaborate internal phragmocone, belemnites could not compress their mantle cavity for jet propulsion to the same extent as the coleoid forms with a much more reduced internal shell. Similar patterns have been inferred from the Pacific fossil record in Japan [42], suggesting a dramatic turnover in particular approximately 100 Ma (figure 3).

Decabrachian coleoids are nektonic predators with streamlined morphology, high metabolic rates and shoaling...
behaviour; adaptations in common with teleost fishes [43]. The majority of modern teleost groups radiated during the Jurassic and Cretaceous [44], concomitantly with the origin of most modern coleoids as revealed by our molecular estimates and the fossil record. The scenario in which Mesozoic ecological shifts are exhibited in teleost fishes, chondrichthyanas (sharks and rays), and shelled invertebrates as investigated by Vermeij [10] can be extended to cephalopods (figure 4). In the face of high-metabolism, robust predators and niche-competitors, the cephalopods may have responded in kind to these evolutionary pressures. We hypothesize that the cephalopods evolved into the forms we are familiar with today, while shelled groups fell into extinction owing to the shifts in predation in this time period. The Mesozoic Marine Revolution can thus be viewed as the final stage in the shift from Palaeozoic ecologies into the modern structure of marine ecosystems, where (at least in the nektonic realm), agility superseded passive defence.

Ammonoids are stem group coleoids, which were common throughout the Late Palaeozoic until the end of the Mesozoic. Evidence from their radula morphology [23,46] suggests that ammonoids primordially had stout teeth, similar to macrophagous predatory cephalopods. In the Jurassic, the group evolved an enlarged calcareous lower jaw (aptychus) and longer, multicuspidate radula teeth, which has been attributed to a shift into microphagous suspension feeding [23,47]. As such, the group ‘stepped out’ of the arms race and ecological competition with the macrophagous predatory coleoids, fishes and marine reptiles during the Jurassic and Cretaceous. The group evolve increasingly ornamented shells in response to increased predation, as revealed from shell repair scar frequency [48], but eventually became extinct at the end of the Cretaceous.

5. Conclusion

Taken together, molecular divergence times and the cephalopod fossil record are consistent with a scenario in which predator–prey arms races shaped the coleoid body plan, biodiversity and ecology. The coincidence with the evolution of jawed vertebrates and teleost fishes during the Devonian Nekton Revolution and the Mesozoic Marine Revolution, suggests that nektonic marine vertebrates have been key antagonists towards cephalopodso throughout most of their evolution.

Data accessibility. All sequence information archived on NCBI, see the electronic supplementary material for accessions. All matrices available on Dryad, doi:10.5061/dryad.180nbh [49].

Authors’ contributions. J.V. carried out all RNA extractions and arranged sequencing procedures in Bristol. A.R.T. carried out all bioinformatics analysis, authored the text, and produced figures. J.V. and D.P. contributed to experimental design and authored the text. I.E.W., A.M.R., K.M.K., R.R.d.F., K.M.H., T.H.O., M.T.F.G., F.D. and M.S.P. contributed sequence data and content on the text.

Competing interests. The authors declare no competing interests.

Funding. J.V. and D.P. are supported by NERC BETR grant NE/P013643/1. Sequencing was supported by a start-up funds from the University of Bristol to J.V. A.R.T. was funded by the University of Bristol Scholarship in Teaching and Research programme.

Acknowledgements. A.R.T. thanks Callum Wright of UoB HPC facility, and Luke Parry, Joseph Keating and James Fleming for valuable discussion. We also thank the late Hans G. Hansson for identification of Bathypolypus arcticus.

References

1. Mather JA. 2008 Cephalopod consciousness: behavioural evidence. Conscious. Cogn. 17, 37–48. (doi:10.1016/j.concog.2006.11.006)
2. Wells MJ, O’Dor RK. 1991 Jet propulsion and the evolution of the cephalopods. Bull. Mar. Sci. 49, 419–432.
3. Mather JA, Kuba MJ. 2013 The cephalopod specialties: complex nervous system, learning, and cognition. Can. J. Zool. 91, 431–449. (doi:10.1139/cjz-2013-0009)
4. Yochelson EL, Flower RH, Webers GF. 1973 The evolutionary implications of the new Late Cambrian monoplacophoran genus Knightocochus upon the origin of the Cephalopoda. Lethaia 6, 275–309. (doi:10.1111/j.1502-3931.1973.tb01199.x)
5. Vinther J, Sperling EA, Briggs DEG, Peterson KJ. 2012 A molecular palaeoecological hypothesis for the origin of aplacophoran molluscs and their derivation from chiton-like ancestors. Proc. R. Soc. B 279, 1259–1268. (doi:10.1098/rspb.2011.1773)
6. Mutvei H, Zhang Y-B, Dunca E. 2007 Late Cambrian monocraonidian nautiloids and their role in cephalopod evolution. Palaeontology 50, 1327–1333. (doi:10.1111/j.1475-4983.2007.00708.x)
7. Krieger B. 2005 Adaptive evolution in Palaeozoic coiled cephalopods. Palaeobiology 31, 253–268. (doi:10.1666/0094-8373(2005)031<0253:SEPCCI>2.0.CO;2)
8. Boyle P, Rodhouse P. 2008 Cephalopods: ecology and fisheries. New York, NY: Wiley.
9. O’Dor RK, Webber DM. 1986 The constraints on cephalopods: why squid aren’t fish. Can. J. Zool. 64, 1591–1605. (doi:10.1139/cjz-1986-241)
10. Vermeij GJ. 1977 The Mesozoic marine revolution: evidence from snails, predators and grazers. Paleobiology 3, 245–258. (doi:10.1017/S0094837300005352)
11. Vermeij GJ. 1987 Evolution and escalation: an ecological history of life. Princeton, NJ: Princeton University Press.
12. Strugnell J, Nishiguchi MK. 2007 Molecular phylogeny of coleoid cephalopods (Mollusca: Cephalopoda) inferred from three mitochondrial and six nuclear loci: a comparison of alignment, implied alignment and analysis methods. J. Mollusc. Stud. 73, 399–410. (doi:10.1093/mollus/eym038)
13. Fuchs D, Iba Y, Tischlinger H, Kreupp H, Klug C. 2015 The locomotion system of Mesozoic Coleoidea (Cephalopoda) and its phylogenetic significance. Lethaia 48, 433–454. (doi:10.1111/let.12155)
14. Clements T, Colleary C, De Baets K, Vinther J. 2016 Buoyancy mechanisms limit preservation of coleoid cephalopod soft tissues in Mesozoic Lagerstätten. Palaeontology 60, 1–14. (doi:10.1111/pal.12267)
15. Klug C, Schweigert G, Fuchs D, Kruta I, Tischlinger H. 2016 Adaptations to squid-style high-speed swimming in Jurassic belemnites. Biol. Lett. 12, 20150877. (doi:10.1098/rsbl.2015.0877)
16. Lindgren AR, Pankey MS, Hochberg FG, Oakley TH. 2012 A multi-gene phylogeny of Cephalopoda supports convergent morphological evolution in association with multiple habitat shifts in the marine environment. BMC Evol. Biol. 12, 129. (doi:10.1186/1471-2148-12-129)
17. Strugnell J, Jackson J, Drummond AJ, Cooper A. 2006 Divergence time estimates for major cephalopod groups: evidence from multiple genes. Cladistics 22, 89–96. (doi:10.1111/j.1096-0031.2006.00066.x)
18. Doguzhaeva LA, Mapes RH, Mutvei H. 1999 A Late Carboniferous spiralid coleoid from the Southern Mid-Continental (USA). In Advancing research on living and fossil cephalopods (eds F Olóriz, FJ Rodríguez-Tovar), pp. 47–57. New York, NY: Springer.
19. Kulessendj J, Doyle P. 2000 Pohlesia mazonensis, an early ‘octopus’ from the carboniferous of Illinois, USA. Paleontology 43, 919–926. (doi:10.1111/1475-4983.00155)
20. Kroger B, Vinther J, Fuchs D. 2011 Cephalopod origin and evolution: a congruent picture emerging from fossils, development and molecules. Bioessays 33, 602–613. (doi:10.1002/bies.201000001)
21. Wamke KM, Meyer A, Eberle B, Lieb B. 2011 Assessing divergence time of Spirula and Sepia (Cephalopoda) based on hemocyanin sequences. Mol. Phylogenet. Evol. 58, 390–394. (doi:10.1016/j.ympev.2010.11.024)
22. Kröger B, Maps RH. 2007 On the origin of bactritoids (Cephalopoda). Palaeontol. Z. 81, 316 – 327. (doi:10.1007/BF02990181)

23. Klug C, Kom D, De Baets K, Kruta I, Maps RH. 2015 Devonian ancestors of Nautilus. Palaeontol. Z. 66, 81 – 98. (doi:10.1007/BF02969479)

24. Dzik J, Kom D. 1992 Devonian ancestors of Nautilus. Palaeontol. Z. 81, 316 – 327. (doi:10.1007/BF02990181)

25. Klug C, Kroeger B, Kirsiing W, Mullins GL, Servais T, Ruiz-Elizondo B, Rucker M. 2016 Resolution of ray-finned fish phylogeny and timing of diversification. Proc. Natl. Acad. Sci. USA 109, 13698 – 13703. (doi:10.1073/pnas.1206625109)

26. Klug C, Kroeger B, Kirsiing W, Mullins GL, Servais T, Ruiz-Elizondo B, Rucker M. 2016 Resolution of ray-finned fish phylogeny and timing of diversification. Proc. Natl. Acad. Sci. USA 109, 13698 – 13703. (doi:10.1073/pnas.1206625109)

27. Bush AM, Bamhach RK. 2011 Paleoecological megatrends in marine Metazoan. Annu. Rev. Earth Planet. Sci. 39, 241 – 269. (doi:10.1146/annurev-earth-040809-152556)

28. Lepage T, Bryant D, Philippe H, Lartillot N. 2007 A general comparison of relaxed molecular clock models. Mol. Biol. Evol. 24, 19 – 30. (doi:10.1093/molbev/msh013)

29. Le SQ, Gascuel O. 2008 An improved general amino acid replacement matrix. Mol. Biol. Evol. 25, 1307 – 1320. (doi:10.1093/molbev/msn067)

30. Miller KG, Near TJ, Eytan RI, Dornburg A, Kuhn KL, Moore JA, Davis MP, Wainwright PC, Friedman M, Smith WL. 2012 Resolution of ray-finned fish phylogeny and timing of diversification. Proc. Natl. Acad. Sci. USA 109, 13698 – 13703. (doi:10.1073/pnas.1206625109)

31. Le SQ, Gascuel O. 2008 An improved general amino acid replacement matrix. Mol. Biol. Evol. 25, 1307 – 1320. (doi:10.1093/molbev/msn067)

32. Lindgren AR. 2010 Molecular phylogenetic relationships among Decapodiformes (Mollusca: Cephalopoda) with special focus on the squids of order Oegopsida. Mol. Phylogenet. Evol. 56, 77 – 90. (doi:10.1016/j.ympev.2010.03.025)

33. Packard A. 1972 Cephalopods and fish: the limits of convergence. Biol. Rev. Camb. Philos. Soc. 47, 241 – 307. (doi:10.1111/j.1469-185X.1972.tb00975.x)

34. Packard A. 1972 Cephalopods and fish: the limits of convergence. Biol. Rev. Camb. Philos. Soc. 47, 241 – 307. (doi:10.1111/j.1469-185X.1972.tb00975.x)

35. Klug C, Kom D. 1992 Devonian ancestors of Nautilus. Palaeontol. Z. 81, 316 – 327. (doi:10.1007/BF02990181)

36. Klug C, Kom D. 1992 Devonian ancestors of Nautilus. Palaeontol. Z. 81, 316 – 327. (doi:10.1007/BF02990181)

37. Kruta I, Landman NH, Maps R, Pradel A. 2014 New insights into the buccal apparatus of the Goniatitina: palaeobiological and phylogenetic implications. Lethaia 47, 38 – 48. (doi:10.1111/let.12036)

38. Kruta I, Landman NH, Rousset I, Cecca E, Tafforeau P. 2011 The Phanerozoic record of global sea-level change. Science 331, 1293 – 1298. (doi:10.1126/science.1198793)

39. Miller KG, Near TJ, Eytan RI, Dornburg A, Kuhn KL, Moore JA, Davis MP, Wainwright PC, Friedman M, Smith WL. 2012 Resolution of ray-finned fish phylogeny and timing of diversification. Proc. Natl. Acad. Sci. USA 109, 13698 – 13703. (doi:10.1073/pnas.1206625109)

40. Iba Y, Mutterlose J, Tanabe K, Sano S-I, Misaki A, Tesabe K. 2011 Belemnite extinction and the origin of modern cephalopods 35 m.y. prior to the Cretaceous — Paleogene event. Geology 39, 483 – 486. (doi:10.1130/G31724.1)

41. Iba Y, Mutterlose J, Tanabe K, Sano S-I, Misaki A, Tesabe K. 2011 Belemnite extinction and the origin of modern cephalopods 35 m.y. prior to the Cretaceous — Paleogene event. Geology 39, 483 – 486. (doi:10.1130/G31724.1)

42. Iba Y, Mutterlose J, Tanabe K, Sano S-I, Misaki A, Tesabe K. 2011 Belemnite extinction and the origin of modern cephalopods 35 m.y. prior to the Cretaceous — Paleogene event. Geology 39, 483 – 486. (doi:10.1130/G31724.1)