PNAS COMMENTARY

Robust resolved phylogeny of hexapods' four classes

Dany Azar[a,b,1]

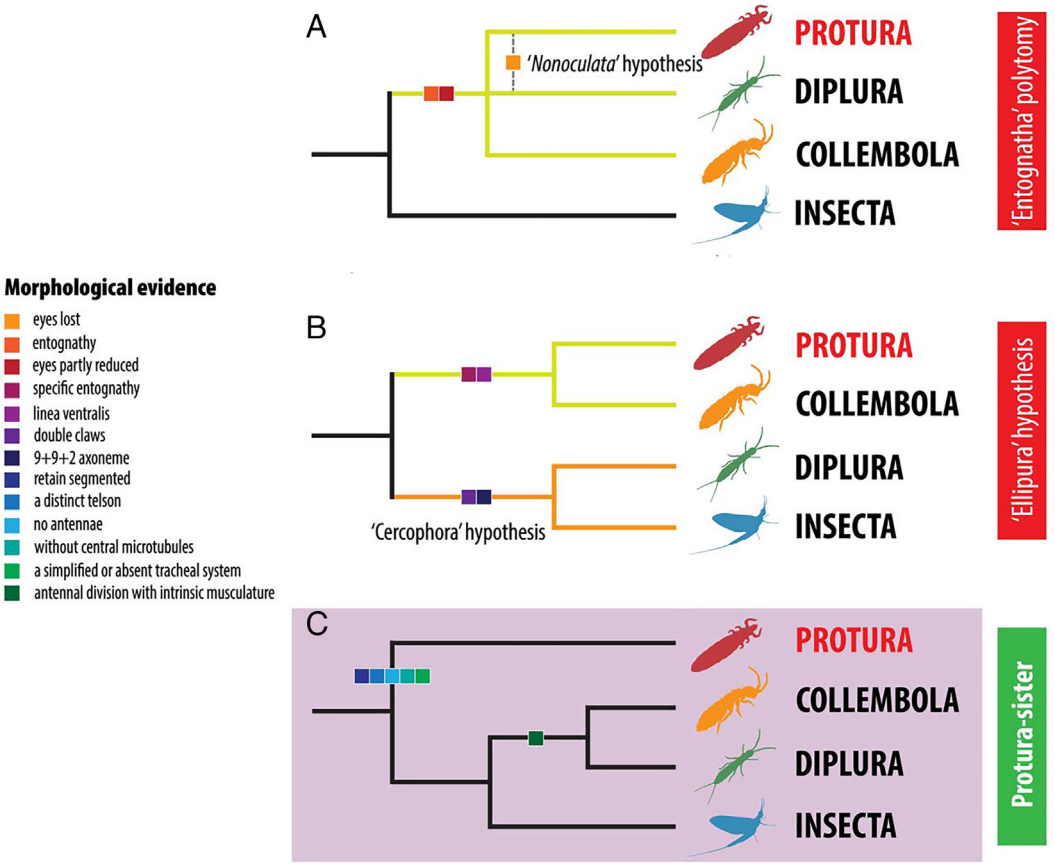

Fig. 1. Main classical phylogenetic tree topologies corresponding to the hypotheses of the evolution of hexapods. (*A*) Entognatha polytomy model; (*B*) Ellipura hypothesis model; (*C*) Protura-sister model proposed by Du et al.

In PNAS, Du et al. (1) interrogated the interrelationships between the main groups of hexapods (six-legged arthropods) and presented a hypothesis to address the problem of insect origins and hexapod diversification by increasing taxon and gene sampling of overlooked basal hexapod groups. Arthropoda are a phylum comprising four major groups: Chelicerata, Crustacea, Myriapoda, and Hexapoda (2). Recent molecular phylogenetic analyses have revealed that Hexapoda and Crustacea form a common clade, the Pancrustacea (3–7), rejecting the traditional grouping of Atelocerata (=Antennata or Tracheata *sensu* Pocock 1893) encompassing Hexapoda with Myriapoda (8). Hexapoda are the largest clade of arthropods and includes the huge biodiverse crown group class Insecta (true insects), along with the considerably smaller class Entognatha [accounting for less than 1% of animal diversity with about 11,000 species described to date (9–11)], which includes the three groups of wingless arthropods that were once considered insects: Collembola (springtails), Protura (coneheads), and Diplura (two-pronged bristletails). Insects and their six-legged relatives are known to comprise more than half of all described species and dominate terrestrial and freshwater ecosystems (12). Understanding the factors behind the success and vast diversity of insects is hindered by incomplete knowledge of their evolutionary origins. A crucial piece of this puzzle lies with the noninsect hexapods, the closest living relatives of insects.

The phylogeny of hexapods has long been debated, with various early evolutionary scenarios proposed. However, these efforts failed to produce a consensus or significant

Author affiliations: [a]State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, China; and [b]Faculty of Science II, Natural Sciences Department, Lebanese University, Fanar-El-Matn 90656, Lebanon

Author contributions: D.A. wrote the paper.

The author declares no competing interest.

See companion article, "Revisiting the four Hexapoda classes: Protura as the sister group to all other hexapods," 10.1073/pnas.2408775121.

[1]Email: danyazar@ul.edu.lb.

Published October 21, 2024.

advancements until recently, when phylogenomics resolved many longstanding discrepancies. A decade ago, an extensive serious phylogenomic study based on transcriptomes was realized (6) and provided the basis of our contemporary consensus on the phylogeny of insects. Nevertheless, this 10-y-old study and several others that followed were focused on the evolutionary relationships within the Insecta but not among basal noninsect Hexapoda groups. Although phylogenomics is increasingly applied to the study of insect phylogeny, this approach can face challenges, such as the high demand for computational resources and systematic errors stemming from large molecular datasets.

> **"In PNAS, Du et al. interrogated the interrelationships between the main groups of hexapods (six-legged arthropods) and presented a hypothesis to address the problem of insect origins and hexapod diversification by increasing taxon and gene sampling of overlooked basal hexapod groups."**

In this issue of PNAS, Du et al. (1) collected field samples of Protura and Diplura, purified them through rearing in the lab, and extracted RNA, ultimately successfully acquiring the transcriptomes for a tiny proturan (with a total body size of about 0.6 mm) and two dipluran species. Usually sampling and sequencing the genomes of miniscule hexapods of basal noninsect groups presents numerous severe technical and financial difficulties and challenges, thus obtaining their transcriptome data is very difficult. Finally, they used 1,013 single-copy orthologous genes to resolve the evolutionary relationships within the four Hexapoda classes.

In their analyses, Du et al. fully considered the systematic errors generated by "big-data-based" and precisely identified potential factors causing inconsistencies in topology through extensive sensitivity tests (also known as loci filtering or gene properties detection), thereby improving the accuracy of tree building. Additionally, they employed different phylogeny strategies (such as multi-species coalescent and concatenation-based) and considered various phylogenetic models (from simple site-homogeneous models to complex site-heterogeneous models) to construct reliable phylogenetic trees. Finally, they conducted comparative analyses on different topologies based on statistical indicators.

Classically several topologies were proposed for the phylogenetic trees reflecting the hypotheses of the evolution of hexapods (Fig. 1), like for instance, the "Entognatha" polytomy model (embracing the "Nonoculata" hypothesis which aligns Protura and Diplura as sister groups), supported by some authors (4, 13, 14); the most popular "Ellipura" hypothesis model that support the "Cercophora" hypothesis (which combines Diplura and Insecta) defended by several workers (6, 7, 15); and the model presented by the ultimate finding of Du et al. which presents a rigorous hypothesis that Protura is the earliest-diverging hexapod lineage ("Protura-sister") (16) and Collembola as a sister group to Diplura, a clade corresponding to the original composition of Entognatha, both supported by high node support values making the finding strongly robust and convincing. It is noteworthy that this model was first proposed by Stummer-Traunfels in 1891 (17). This conclusion was recovered in almost all complex site-heterogeneous models (PMSF, CAT-GTR) and was also supported by coalescent-based and simple site-homogeneous models (LG, GTR) when considering the appropriate genes (e.g., gene with higher evolutionary rate). It has been demonstrated recently that the site-heterogeneous models can successfully eradicate the effect of the problematic long branch attraction artifact (LBA) (18) which is systematic error where distantly related lineages are incorrectly inferred to be closely related. In their proposed phylogeny, Du et al. bring molecular tools in support of some morphological evidence where Collembola and Diplura present similar mouthpart structures and musculature (19, 20).

In this PNAS issue, the authors of the "A new story of the four Hexapoda classes: Protura as the sister group to all other hexapods"(1) not only presented a good, well-designed, and substantial analysis yielding a robust phylogeny of the four Hexapoda classes and emphasize that appropriate models and genes can enhance the accuracy of the phylogenetic trees, but also displayed the results artistically in nice-looking illustrations. They came to an important conclusion that Protura are the sister group to all other hexapods, and this implies that the last common hexapod ancestor was terrestrial and possessed complex adaptations for life on land. I believe that the work of Du et al. is a sound and robust achievement that will constitute undeniably a milestone for future hexapod phylogenies.

1. S. Du *et al.*, Revisiting the four Hexapoda classes: Protura as the sister group to all other hexapods. *Proc. Natl. Acad. Sci. U.S.A.* **121**, e2408775121 (2024).
2. G. Sasaki, K. Ishiwata, R. Machida, T. Miyata, Z.-H. Su, Molecular phylogenetic analyses support the monophyly of Hexapoda and suggest the paraphyly of Entognatha. *BMC Evol. Biol.* **13**, 236 (2013).
3. G. Giribet, G. D. Edgecombe, W. C. Wheeler, Arthropod phylogeny based on eight molecular loci and morphology. *Nature* **413**, 157–161 (2001).
4. J. C. Regier *et al.*, Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. *Nature* **463**, 1079–1083 (2010).
5. B. M. von Reumont *et al.*, Pancrustacea phylogeny in the light of new phylogenomic data: Support for Remipedia as the possible sister group of Hexapoda. *Mol. Biol. Evol.* **29**, 1031–1045 (2012).
6. B. Misof *et al.*, Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346**, 763–767 (2014).
7. R. G. Beutel, M. I. Yavorskaya, Y. Mashimo, M. Fukui, K. Meusemann, The phylogeny of Hexapoda (Arthropoda) and the evolution of megadiversity. *Proc. Arthropod. Embryol. Soc. Jpn.* **51**, 1–15 (2017).
8. O. Kraus, M. Kraus, Phylogenetic system of the Tracheata (Mandibulata): On "Myriapoda": Insecta interrelationships, phylogenetic age and primary ecological niches. *Verh. Natwiss. Ver. Hambg.* **34**, 5–31 (1994).
9. A. Szeptycki, Catalog of the world Protura. *Acta Zool. Cracov. Ser. B* **50**, 1–210 (2007).
10. A. Potapov *et al.*, Towards a global synthesis of Collembola knowledge–Challenges and potential solutions. *Soil Org.* **92**, 161–188 (2020).
11. A. Sendra, A. Jiménez-Valverde, J. Selfa, A. S. Reboleira, Diversity, ecology, distribution and biogeography of Diplura. *Insect Conserv. Divers.* **14**, 415–425 (2021).
12. J. L. Rainford, M. Hofreiter, D. B. Nicholson, P. J. Mayhew, Phylogenetic distribution of extant richness suggests metamorphosis is a key innovation driving diversification in insects. *PLoS One* **9**, e109085 (2014).
13. W. Hennig, *Die Stammesgeschichte der Insekten* (Waldemar Kramer, ed. 1, 1969).
14. C. Bitsch, J. Bitsch, Phylogenetic relationships of basal hexapods among the mandibulate arthropods: A cladistic analysis based on comparative morphological characters. *Zool. Scripta* **33**, 511–550 (2004).
15. G. Giribet, G. D. Edgecombe, J. M. Carpenter, C. A. D'Haese, W. C. Wheeler, Is Ellipura monophyletic? A combined analysis of basal hexapod relationships with emphasis on the origin of insects. *Org. Divers. Evol.* **4**, 319–340 (2004).
16. J. Lozano-Fernandez *et al.*, Pancrustacean evolution illuminated by taxon-rich genomic-scale data sets with an expanded remipede sampling. *Genome Biol. Evol.* **11**, 2055–2070 (2019).

17. R. R. von Stummer-Traunfels, Vergleichende Untersuchungen über die Mundwerkzeuge der Thysanura und Collembolen. *Sitzungsberichte der kaiserlichen Akademie der Wissenschaften: Mathematisch-Naturwissenschaftliche Classe. Abtheilung I* **100**, 216–234 (1891).
18. N. Lartillot, H. Brinkmann, H. Philippe, Suppression of long-branch attraction artefacts in the animal phylogeny using a site-heterogeneous model. *BMC Evol. Biol.* **7**, S4 (2007).
19. A. Blanke *et al.*, Structural mouthpart interaction evolved already in the earliest lineages of insects. *Proc. R. Soc. B* **282**, 20151033 (2015).
20. A. Blanke, R. Machida, The homology of cephalic muscles and endoskeletal elements between Diplura and Ectognatha (Insecta). *Org. Divers. Evol.* **16**, 241–257 (2016).